ADD 19 1926

THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

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VOL. LXXVI—No. 2

Issued April 1, 1926

BALTIMORE, U. S. A.

1926

Entered as second-class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 5, 1918

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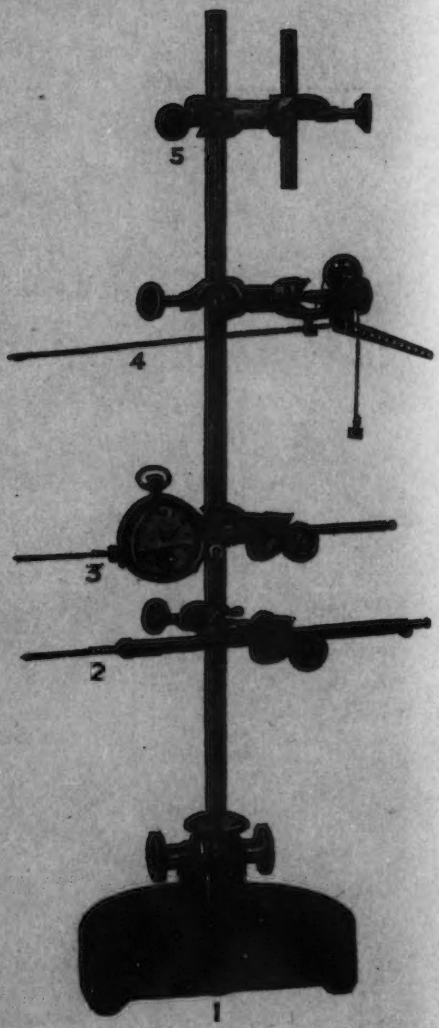

JOSEPH BECKER

437 West 59th Street **New York City**

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 76 APRIL 1, 1926 No. 2

THE NUTRITIVE VALUE OF VARIOUS LAYERS OF THE WHEAT AND CORN KERNEL

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Received for publication November 14, 1925

It has been known for some that the various layers of the cereal grains, with a few exceptions, have their vitamines localized in the peripheral layers and the germ. Little is known, however, concerning the distribution of proteins and inorganic salts. One of the first papers on the subject was published by one of us (1); it dealt with the chemical composition of the various layers of corn originating in South Africa. It is not difficult to deduce from this paper that the layer containing the pericarp and the germ has a high nutritive value. Somewhat similar work on corn of the same origin has been published by Juritz (2) and McCrea (3). Cross and Swain (4) have confined their attention to wheat. They have isolated a number of proteins and determined their amino-acid content by the Van Slyke method. Their work shows that the two main protein constituents, gliadin and glutenin, differ very considerably in their content of amino-acids, to which the biological value of the protein is usually ascribed. For example, glutenin contains more tryptophane, histidine, arginine, lysine and tyrosine, and little less cystine than gliadin. From data such as this (and other data which we need not quote here) it is likely that the various milled products of the cereal grain would show not only differences in vitamine content but also in protein content. To study this question, we have subjected the milling fractions of wheat and corn[1] to a fractionation process in order to determine their content of a number of proteins. At the same time, the biological value of the milled products has been determined according to the method devised by Mitchell (5), rats being the experimental animals used.

[1] These samples were kindly supplied to us by Washburn-Crosby Co.

TABLE 1

RAT NUMBER	INITIAL WEIGHT	FINAL WEIGHT	WEEKLY FOOD INTAKE	WEEKLY NITROGEN INTAKE	WEEKLY FECAL NITROGEN	WEEKLY URINARY NITROGEN	METABOLIC NITROGEN IN FECES PER GRAM FOOD	ENDOGENOUS NITROGEN IN URINE PER 100 GRAMS BODY WEIGHT	FOOD NITROGEN IN FECES	ABSORBED NITROGEN	FOOD NITROGEN IN URINE	TOTAL FOOD NITROGEN RETAINED	BIOLOGICAL VALUE
	grams	*grams*	*grams*	*mgm*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Period 1. Protein-free ration													
1	118	106	57.9	17.4	45.5	229.3	0.79	205					
2	117	104	51.8	15.5	32.7	281.1	0.63	254					
3	116	102	55.6	16.7	22.1	279.7	0.40	257					
4	124	110	54.4	16.3	28.0	210.6	0.50	180					
Period 2. Bran ration													
1	110	113	63.2	856.4	302.5	476.7			245.9	610.5	249.2	361.3	59
2	108	108	55.0	745.3	300.1	443.1			268.8	476.5	181.7	294.8	62
3	110	112	68.8	932.2	273.7	531.6			227.5	704.7	261.9	442.8	63
4	113	119	62.0	840.1	290.4	465.2			239.6	600.5	257.6	342.9	57
Period 3. Standard middlings ration													
1	122	131	79.2	1086.0	457.6	515.2			378.1	707.9	258.4	449.5	63
2	107	117	67.3	922.7	364.1	490.7			296.1	626.6	233.1	393.5	63
3	121	131	81.3	1115.0	482.6	539.8			422.4	692.6	251.3	441.3	64
4	124	135	78.1	1071.0	444.7	471.0			378.8	692.2	240.5	451.7	65
Period 4. Red dog ration													
1	130	143	80.9	1094.0	288.6	480.3			198.6	895.4	204.6	690.8	77
2	115	128	69.8	922.8	246.4	442.4			161.9	760.9	177.6	583.3	77
3	130	139	74.2	980.9	251.5	446.6			184.0	796.9	157.4	639.5	80
4	137	152	84.7	1120.0	404.9	492.1			318.9	801.1	234.9	566.2	71
Period 5. Second clear ration													
1	140	133	50.8	693.4	119.9	559.2			57.8	635.6	284.8	350.8	55
2	129	130	64.5	880.4	170.5	497.2			79.6	800.8	231.7	569.1	71
3	139	140	70.1	956.9	183.6	584.7			107.9	849.0	280.4	568.6	67
4	155	157	77.1	1052.0	211.0	636.4			119.2	932.8	360.3	572.5	61
Period 6. Protein free ration													
1	130	119	48.8	14.6	64.9	248.4	1.33	200					
2	123	109	41.2	12.4	66.3	222.9	1.61	192					
3	133	123	54.9	16.5	68.9	240.6	1.26	188					
4	152	138	59.7	17.9	81.4	254.3	1.36	176					

TABLE 1—*Concluded*

RAT NUMBER	INITIAL WEIGHT	FINAL WEIGHT	WEEKLY FOOD INTAKE	WEEKLY NITROGEN INTAKE	WEEKLY FECAL NITROGEN	WEEKLY URINARY NITROGEN	METABOLIC NITROGEN IN FECES PER GRAM FOOD	ENDOGENOUS NITROGEN IN URINE PER 100 GRAMS BODY WEIGHT	FOOD NITROGEN IN FECES	ABSORBED NITROGEN	FOOD NITROGEN IN URINE	TOTAL FOOD NITROGEN RETAINED	BIOLOGICAL VALUE
	grams	*grams*	*grams*	*mgm*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Period 7. First clear													
1	117	108	27.0	372.3	55.0	398.8			19.7	352.6	176.0	176.6	50
2	108	106	34.6	477.1	100.4	410.0			43.7	433.4	209.9	223.5	52
3	122	122	44.3	610.9	112.4	451.9			54.9	556.0	231.1	324.9	58
4	137	126	32.0	441.3	101.8	436.3			57.1	384.2	212.7	171.5	45
Period 8. Patent flour													
1	107	107	42.1	546.7	60.3	443.7			6.0	540.7	234.0	306.7	57
2	105	105	41.3	536.1	78.7	396.0			9.3	526.8	204.9	321.9	61
3	120	120	49.3	640.2	87.6	481.2			22.1	618.1	272.4	345.7	56
4	130	124	44.4	576.4	88.8	418.5			24.5	551.9	310.2	241.7	44
Period 9. Corn feed meal													
1	114	117	55.1	620.8	158.1	400.1			88.7	532.1	176.0	356.1	67
2	111	124	72.1	812.3	219.4	475.1			96.1	716.2	267.1	449.1	63
3	126	134	71.7	807.8	203.7	535.8			105.5	702.3	320.0	382.3	54
4	131	139	73.5	828.1	214.5	528.3			94.2	733.9	315.0	418.9	57
Period 10. Corn meal													
1	117	105	34.4	360.3	73.3	379.7			30.6	329.7	166.6	163.1	49
2	124	117	41.3	429.8	84.5	344.2			12.2	417.6	136.9	280.7	67
3	136	134	54.5	566.8	79.3	453.3			3.0	563.8	238.7	325.1	58
4	144	128	50.9	532.3	194.5	417.9			116.1	416.2	211.2	205.0	49
Period 11. Protein-free ration													
1	108	102	40.1	12.0	48.8	199.0	1.22	190					
2	111	102	36.3	10.9	64.5	178.0	1.78	167					
3	126	117	39.2	11.8	56.4	185.0	1.44	152					
4	130	119	45.4	12.6	71.6	181.0	1.58	146					

The results obtained by us are not in accord with those obtained by Miss Hartwell (6) who, studying the comparative nutritive value of white and whole wheat bread, claims that the former is quite sufficient to maintain the health and vigor of rats.

Our experiments tend to show that even using the vitamines and vita-

TABLE 2

RAT NUMBER	INITIAL WEIGHT	FINAL WEIGHT	WEEKLY FOOD INTAKE	WEEKLY NITROGEN INTAKE	WEEKLY FECAL NITROGEN	WEEKLY URINARY NITROGEN	METABOLIC NITROGEN IN FECES PER GRAM FOOD	ENDOGENOUS NITROGEN IN URINE PER 100 GRAMS BODY WEIGHT	FOOD NITROGEN IN FECES	ABSORBED NITROGEN	FOOD NITROGEN IN URINE	TOTAL FOOD NITROGEN RETAINED	BIOLOGICAL VALUE
Period 1. Protein-free ration													
	grams	*grams*	*grams*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
5	125	116	56.3	17.0	60.4	261.3	1.07	217					
6	111	107	56.6	17.0	63.1	191.9	1.11	176					
9	136	123	71.0	21.3	77.8	234.2	1.10	181					
10	100	89	50.3	15.1	54.5	214.2	1.08	227					
Period 2. Corn meal													
5	120	111	50.6	531.3	121.5	478.1			62.3	469.0	235.6	233.4	50
6	111	90	28.0	294.0	89.8	448.7			54.5	239.5	269.8	30.3	0
9	127	124	60.5	645.3	232.9	592.2			160.9	474.4	358.8	115.5	24
10	92	85	42.0	441.0	208.4	464.1			162.8	278.2	231.1	17.1	6
Period 3. Corn feed meal													
5	116	125	69.6	800.4	188.8	476.7			100.4	700.0	230.9	469.1	67
6	105	111	71.3	820.0	259.2	518.7			156.5	563.5	323.2	340.3	51
9	113	122	67.4	775.1	178.7	421.4			92.4	682.7	197.0	485.7	71
10	87	87	59.6	685.4	199.2	454.3			122.9	562.5	258.0	303.9	54
Period 4. Patent flour ration													
5	120	120	46.5	582.6	92.6	473.1			28.4	554.2	236.7	317.5	49
6	106	87	23.9	299.5	57.9	400.8			18.9	280.6	224.2	56.4	20
9	119	110	42.4	531.3	110.1	460.1			52.4	478.9	236.8	242.1	51
10	91	82	32.1	402.2	70.7	425.6			34.7	367.5	232.7	134.8	37
Period 5. First clear ration													
5	122	125	54.0	680.4	112.2	508.3			32.3	648.1	272.4	375.7	58
6	91	90	43.9	553.1	149.1	473.1			69.9	483.2	304.8	178.4	37
9	112	113	52.4	660.2	99.2	532.1			23.2	637.0	307.1	329.9	52
10	84	80	35.9	452.3	72.1	424.9			31.5	420.8	242.9	177.9	42
Period 6. Protein-free ration													
5	119	111	44.9	13.5	70.8	211.3	1.58	184					
6	85	80	43.1	12.9	86.2	154.9	2.00	188					
9	110	101	47.7	14.3	73.6	216.0	1.54	205					
10	76	71	39.1	11.7	44.5	162.4	1.14	221					

TABLE 2—*Concluded*

RAT NUMBER	INITIAL WEIGHT	FINAL WEIGHT	WEEKLY FOOD INTAKE	WEEKLY NITROGEN INTAKE	WEEKLY FECAL NITROGEN	WEEKLY URINARY NITROGEN	METABOLIC NITROGEN IN FECES PER GRAM FOOD	ENDOGENOUS NITROGEN IN URINE PER 100 GRAMS BODY WEIGHT	FOOD NITROGEN IN FECES	ABSORBED NITROGEN	FOOD NITROGEN IN URINE	TOTAL FOOD NITROGEN RETAINED	BIOLOGICAL VALUE
	Period 7. Second clear ration												
	grams	*grams*	*grams*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
5	115	114	38.4	549.5	66.3	347.0			3.3	546.2	151.2	395.0	72
6	83	94	55.3	791.3	173.1	358.5			62.5	728.8	193.9	534.9	73
9	102	105	50.8	727.0	111.3	383.9			35.1	691.9	184.1	507.8	73
10	66	75	39.9	571.0	78.4	330.6			30.5	540.5	178.3	362.2	67
	Period 8. Red dog ration												
5	124	138	69.2	947.3	322.6	377.5			205.0	742.3	170.5	571.8	7
6	98	110	73.0	999.3	376.1	326.9			230.1	769.2	136.6	632.6	82
9	123	145	97.0	1328.0	381.3	469.7			238.8	1089.0	228.5	860.5	79
10	85	108	76.1	1042.0	215.7	443.8			119.9	922.1	240.2	681.9	74
	Period 9. Standard middlings ration												
5	137	136	55.2	711.0	269.9	440.3			171.1	539.9	260.1	279.8	52
6	115	125	57.7	113.0	482.2	516.8			307.7	822.3	299.6	522.7	64
9	144	138	74.9	964.7	439.2	559.6			332.1	632.6	322.7	309.9	49
10	106	110	61.5	792.1	345.5	415.8			263.7	528.4	192.2	336.2	64
	Period 10. Bran ration												
5	137	134	53.4	677.6	234.3	430.3			136.0	541.6	269.1	272.5	50
6	128	132	81.9	1039.0	449.7	496.9			286.7	752.3	265.5	486.8	65
9	145	150	88.0	1117.0	530.6	582.1			407.4	709.6	353.7	355.9	50
10	112	113	59.9	760.1	318.4	471.0			235.1	525.0	244.7	280.3	53
	Period 11. Protein-free ration												
5	133	124	47.5	14.2	89.7	135.7	1.89	106					
6	128	109	41.7	12.5	83.2	209.1	1.99	176					
9	142	132	52.2	15.7	70.8	195.5	1.36	143					
10	105	93	35.9	11.8	52.1	194.8	1.45	197					

sterols,[2] known to be necessary for rats, the natural foodstuffs probably contain additional substances, at present unknown to us, which render it practically impossible to determine the biological value of proteins

[2] According to our new proposal of a vitamine nomenclature (Bull. Soc. Chim. Biologique, vii, p. 761, 1925) we have divided the vitamines into two groups: the water-soluble group, to be designated the "vitamine" group, and the fat-soluble group, the "vitasterols."

in a natural foodstuff. To arrive at the biological value of the protein it would be necessary to isolate the various proteins and to feed them together in an artificial food-mixture. We find a wide variation between the nutritive values of the various layers of grain. Those layers which are known to contain most of the vitamines are, from the nutritive point of view, much superior to those containing comparatively little vitamines. The question arises then whether substances of vitamine nature, but still unknown to us, possess the power to increase the biological value of the proteins. To settle this point new experiments are being performed which will be reported on in the near future.

The work on the nutritive value of the milling fractions of wheat and corn was initiated by chemical fractionation of the proteins contained therein. The work consisted in estimating:

1. The total protein content of each of the layers.

2. The fraction soluble in 70 per cent alcohol which represents the gliadins.

3. The fraction soluble in 5 per cent solution of potassium sulphate, representing the globulins; and finally

4. The glutenin fraction, which was estimated by subtracting from the total protein the second and the third fraction.

The amount of gluten which represents the sum of gliadin and glutenin is also given in the table below which represents an average figure of two determinations.

Wheat products (per cent on a dry basis)

	FEED			FLOUR		
	6299B. Bran	6298B. Standard middlings	6297B. Red dog	6296B. Second clear	6295B. First clear	6294B. Patent flour
1. Total protein	15.60	15.91	18.06	16.42	14.69	12.75
2. Alcohol (70 per cent) soluble (gliadin)	2.73	2.53	3.96	6.47	7.38	6.71
3. Potassium sulphate (5 per cent) soluble	5.87	5.49	8.83	4.67	2.47	1.86
4. Glutenin	7.00	7.89	5.27	5.28	4.84	4.18
5. Gluten (gliadin + glutenin)	9.73	10.42	9.23	11.75	12.22	10.89
Per cent of the total protein, dry basis						
Alcohol (70 per cent) soluble	17.50	15.90	21.93	39.40	50.24	52 63
Potassium sulphate (5 per cent) soluble	37.63	34.50	48.89	28.44	16.81	14.59
Glutenin	44.87	49.59	29.18	32.16	32.95	32.78
Gluten	62.37	65.49	51.11	71.56	83.19	85.41
Per cent gliadin in gluten	28.06	24.28	42.90	55.06	60.39	61.62
Per cent glutenin in gluten	71.94	75.72	57.10	44.94	39.61	38.38

Corn products

	CORN MEAL. 6300B. ON A DAY BASIS		CORN FEED MEAL. 6301B. ON A DAY BASIS	
	Per cent	Per cent of the total protein	Per cent	Per cent of the total protein
Total protein	10.38		10.98	
Alcohol (70 per cent) soluble	3.21	30.92	2.43	22.13
Potassium sulphate (5 per cent) soluble	1.35	13.01	2.03	18.49
Glutenin	5.82	56.07	6.52	59.38
Zein + glutenin	9.03	86.99	8.95	81.51

The potassium sulphate soluble fraction represents the globulins. From this table we may conclude:

1. Gliadin is found to a large extent in patent flour, which, however, contains the smallest amount of globulin.

2. In by-products, as in bran, the globulin content is high.

3. The quantity of glutenin diminishes steadily as we proceed from the outer layers to what represents the patent flour.

4. What is true of wheat is true, very largely, of corn. Corn meal is richer in zein (the gliadin of corn) while corn feed meal is richer in gobulin.

In general, we may say that the peripheral layers of the cereal grains contain proteins which are, dietetically speaking, more complete than those to be found in white flour.

The second part of this paper deals with animal experiments on rats to test the conclusions reached by the chemical method.

The animal experiments were on general lines the same as published by Mitchell. The main difference was in the caging of animals which we consider simpler and in many ways more efficient. Instead of filter paper for absorbing the urine of the animal we have used two false wire bottoms one above the other, the coarser wire being the one on which the animal was placed. The mesh of this wire screen was coarse enough to prevent any retention of feces. The second wire screen was the one on which the feces were caught. The urine collected on the bottom of the glass vessel from which it could be washed out quantitatively.

To prevent scattering of the food, the food containers were fitted with tunnels made of sheet iron so that the animal could dip only his head into the container. The general arrangement can be seen on the appended photograph (fig. 1).

In the glass vessel was placed a certain amount of 2 per cent sulfuric acid. The cages were cleaned four times a week, at which time both the screens and the glass vessels were washed with water and filled up to two liters in a measuring flask, from which aliquote samples were taken for

analysis. In diets liable to be scattered, as in the wheat and corn bran, the food remnants, filtered from the urine, were dried in the air and weighed. These amounts were taken into consideration in calculating the amounts of food eaten (4th column of the appended table). The feces were quantitatively removed from the fine screen, dried and aliquots weighed out for analysis.

Diets. The diets used were made out of the various milling fractions which corresponded to about 1.3 per cent of nitrogen. Aside from the cereal fractions, the diets contained 2 per cent of cod liver oil, 7 per cent of butter, 15 per cent of lard and 4 per cent salt mixture prepared according to Osborne and Mendel, the whole made up to 100 per cent with starch. Diets made out of corn contained on an average 1.03 per cent of nitrogen for the corn feed meal and 1.14 per cent nitrogen for the corn meal. The nitrogen

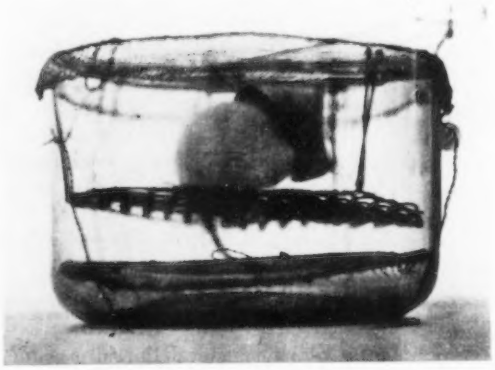

Fig. 1

contents of the diets are given in the 5th column of the table and are not calculated but actually obtained from analysis.

As the source of vitamine B, the animals were given 0.5 cc. of aqueous extract of rice-polishings which corresponded to 1 grain of rice polishings daily. The amount of nitrogen contained therein was 3.5 mgm. per day and was not taken into account.

As regards the arrangement of the experiment, the rats were kept three days on each of the diets without analysis, later the excreta were collected for the subsequent seven days and analyzed.

Discussion of the results. It can be generally stated that the biological value of proteins contained in a natural foodstuff depends on a good many unknown factors. It seems to us that the biological value of proteins in such products can only be accurately determined if the vitamine content is not altered with every change of diet. From our

tables it is entirely clear that the milling fractions of the cereals which contain the peripheral layers *are dietetically very much superior* to those which contain the endosperm. *The total dietetic value* of food cannot be identified with the biological value of its proteins. Animals on inferior diets and which lose weight showed an increased N-secretion and therefore wrong values according to Mitchell's formula. In a word, the biological value depends a good deal on the fact whether the animal is actually gaining or losing weight; and even if the increase in weight is normal, we are not certain that the endogenic nitrogen changes proportionally with the growth.

The determination of the biological value of the proteins yields an absolute figure only when isolated proteins are fed. It gives, however, only a relative value when natural foodstuffs are fed for these depend on the combination of their true dietetic value together with the value of the proteins they contain.

CONCLUSIONS

1. The various layers of the cereal grains differ not only in their vitamine content and in their content of inorganic salts but also in their general protein make-up. The fractions known to contain the bulk of the vitamines contain also proteins of high biological value.

2. The fractions which contain the pericarp and the germ and which serve as the main ingredient of the dietetically superior whole grain flour possess a much higher nutritive value for rats than fractions which are composed mainly of the endosperm (patent flour). It cannot be decided at present whether this superior nutritive value depends on their content in superior proteins or in their vitamines or finally to a combination of both factors.

3. One gains the impression, however, that this superior food value is due to the superiority of the vitamine complex contained in natural foodstuffs as compared to vitamine extracts. The question arises therefore whether certain vitamines are able to increase the biological value of the proteins.

4. The milling fraction of wheat known under the name "red dog" gives the best combination of the highest biological value of the proteins of wheat together with the highest general dietetical value.

5. It is the same "red dog" fraction which shows on chemical examination the highest total protein content and the highest globulin fraction (the fraction soluble in 5 per cent potassium sulphate).

6. As regards corn, the corn feed meal is dietetically much superior to the corn meal. As with wheat, the former fraction is richer in its globulin content.

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THE NATURE AND TIME RELATIONS OF THE COMPRESSION SOUNDS OF KOROTKOV IN MAN

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Received for publication December 18, 1925

A search of the literature reveals but few attempts at objective study of the Korotkov compression sounds. As far as I can discover, Hooker and Southworth (1914) were the first to publish optical records of these sounds in man. Their method was that of Einthoven and Geluk (1894), by which the sound vibrations were received by a microphone, transmitted to a capillary electrometer, and recorded by photographing the movements of the mercury in magnified form. This recording system was made just sensitive enough to register every sound perceptible to the ear. They did not attempt an analysis of the individual sounds. Erlanger (1916) employed the Frank capsule for direct registration of the sounds occurring in a dog's femoral artery during decompression. He states, however, that his recording system was not sensitive enough to register the fainter sounds, and his analysis of the sound vibrations is further open to the criticism that the rubber dam which he used as a recording membrane was probably inadequate to its purpose. This criticism rests upon the careful study of the physical principles involved in direct sound registration made by Wiggers and Dean (1917a, b), who, in order to render the system sufficiently sensitive, found it necessary to employ an extremely thin film of rubber cement as the recording membrane. Bramwell and Hickson (1925) published a few phonoarteriograms which were obtained by the method of Wiggers and Dean (1917a, b), but attempted no analysis of the nature of the vibrations during progressive decompression. In fact, their work was only incidental to a study of the change in form of the pulse wave in the course of transmission.

METHOD. Observations were made upon 29 normal subjects, all of whom were healthy young adults, and upon 5 patients who presented signs of aortic regurgitation.[1] From the large number of records obtained approximately 250 individual sounds and pulses were selected for analysis, and over 1500 measurements of all kinds were made. Time intervals were

[1] These experiments were performed in 1921, publication having been delayed by press of other matters.

determined by means of the Lucas coördinate comparator. In each experiment the subject lay flat on his back. A receiver identical with that used by Wiggers and Dean (1917a) for recording heart sounds from the chest wall was strapped over the brachial artery in the antecubital fossa immediately distal to the cuff. Petrolatum was spread between the skin and the periphery of the receiver to prevent leaks. Sounds were recorded by the direct method, in which the technic developed by Wiggers and Dean (1917a, b) was carefully followed. No correction for parallax was made when two capsules were used to register sound and pulse simultaneously. No correction was made for delay in transmission of the compression pulse from the cuff, inasmuch as this figure was experimentally determined to be inappreciable. The standard Erlanger cuff (effective width 12 cm., length 16 cm.) was employed in most of the experiments. It was held in place by a rigid canvas cover buckled firmly about the arm. A few records were made with a specially constructed narrow cuff having an effective width of 6 cm., and a few with an ordinary commercial cuff. The cuff was inflated from a pressure tank, with manometer control; deflation was accomplished automatically through a needle valve. The compression pulse was recorded through a closed system by an optical capsule covered with rubber dam. This capsule was vertically aligned with the sound-recording capsule. In a few experiments pressure fluctuations within the cuff were transmitted directly to the capsule, so that the beam of light reflected from the mirror of the pulse-recording membrane travelled diagonally across the field. In most of the experiments, however, a sphygmoscope system with Erlanger bulb was introduced between cuff and capsule, and when pulse curves were to be recorded deflation of the cuff was temporarily interrupted. The recording system never failed to respond to an audible compression sound, but it did not discover inaudible sounds.

ANALYSIS OF THE SOUNDS. In comparison with the fundamental heart sounds (cf. Wiggers and Dean, 1917b), the compression sounds of Korotkov are relatively simple in character. Figure 1 is a full-sized reproduction of seven individual sounds, showing the essential details. In analyzing

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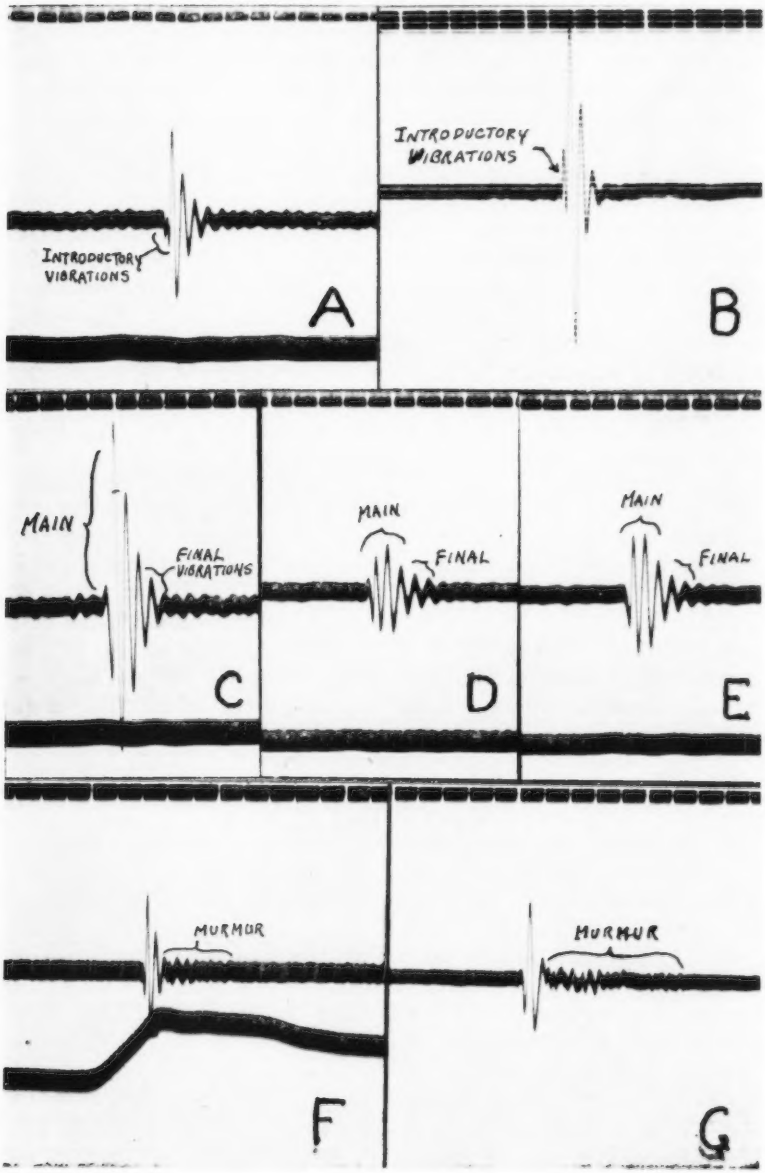
Fig. 1. Seven individual sounds, reproduced full size to show details.

A—shows *introductory* vibrations in an early sound.
B—shows *introductory* vibrations in a late sound.
C—a sound in which the first *main* vibration is the highest.
D—a sound in which the second *main* vibration is the highest.
E—a sound in which the *main* vibrations are of equal amplitude.
F—shows a murmur accompanying the sound, obtained with a cuff of normal width.
G—same subject as F, showing accentuation of the murmur when an unusually narrow cuff is employed.
Time 0.04 second.


INTRODUCTORY VIBRATIONS
A
INTRODUCTORY VIBRATIONS
B
MAIN
FINAL VIBRATIONS
C
MAIN
FINAL
D
MAIN
FINAL
E
MURMUR
F
MURMUR
G

a large number of records it is possible to distinguish three component groups of vibrations, which may be designated as *introductory*, *main*, and *final*, vibrations. The *introductory* vibrations are characterized by their short period, ranging from 0.0039 to 0.01 second (e.g., range in frequency between 256 and 100 per second), and by their relatively small amplitude (fig. 1A, B). There are never more than one or two of these short vibrations in a single sound. Most of them are to be found in the first ten or twelve sounds to appear during decompression. Their presence is chiefly responsible for the fact that the pitch of the early sounds is higher than that of the middle sounds, where they rarely occur. They reappear occasionally in the later sounds, as illustrated by figure 1 B, a sound which was the twenty-eighth to be recorded in a sequence of thirty-eight. In general, the middle sounds are the loudest, and the loudest sounds are characteristically free from introductory vibrations of short period and small amplitude. In this respect my results are not in accord with those of Erlanger (1916), whose records reveal a close relationship between loud sounds and rapid initial vibrations. This point will be discussed later.

The *main* vibrations compose the most conspicuous part of each sound. They are set apart by their relatively long period and great amplitude. The most intense sounds to be recorded, practically without exception, begin abruptly with an oscillation whose period and amplitude are of a magnitude characteristic of main vibrations (fig. 1C). The amplitude of the individual main vibrations varies considerably. Although the first main vibration is usually the one of greatest amplitude (cf. fig. 1C), in certain sounds the second is higher than the first (cf. fig. 1D), and in others the main vibrations are all of nearly equal amplitude (cf. fig. 1E). Here again my findings are not in agreement with Erlanger's (1916), inasmuch as he reports that the first main vibration is always the highest.

Final vibrations are differentiated from main vibrations by their small amplitude. They are well shown in figure 1C, D, E.

My measurements of the Korotkov sounds are summarized in table 1. The sounds are composed of a series of regular vibrations of generically uniform character. The number of vibrations in a single sound varies from three to six. The latter figure is never exceeded, nor does any sound produced by arterial compression contain less than three vibrations, but sounds recorded from the uncompressed artery in patients with aortic regurgitation comprise but two vibrations (cf. table 1, C1X06). The extreme range of vibration period is from 0.0039 to 0.025 second, e.g., from 256 to 40 dv per second. Inasmuch as the established upper limit of 0.025 second was exceeded in less than two and a half per cent of the measurements, I have assumed that vibrations of longer period do not consist purely of sound. Table 1 shows the period range, average period, and average frequency of each vibration. It will be observed that the

average pitch of the last four vibrations is practically constant at a frequency of about 51 dv per second, whereas that of the first and second vibrations is distinctly higher with a frequency of 69 and 55 dv per second, respectively. The disparity in pitch between the first and succeeding vibrations arises from two sources: 1, the first vibration is often an *introductory* vibration whose period does not exceed 0.01 second, and 2, the period of initial *main* vibrations is generally short relative to that of succeeding main vibrations. The extremes of total duration of a single sound are represented by 0.041 second in a sound of 4 double vibrations, and 0.1275 second in a sound of 6 double vibrations. Inasmuch as the shortest sound comprised four, rather than three double vibrations, and inasmuch as the duration of many sounds of 6 double vibrations is less than 0.1275 second, it is apparent that the total duration of a single sound is dependent, not only upon the number of component vibrations, but also upon the duration of component vibration periods.

Table 2 contains a complete analysis of all the sounds recorded in a typical experiment. As this table shows, when decompressing pressure is still high, allowing only a small volume of blood to enter the compressed artery at each beat, the pitch of the compression sounds is relatively high (cf. average periods and frequencies of sounds 1 to 10), but with increasing volume of the penetrating jet of blood the short introductory vibrations disappear (cf. sounds 12, 13, 14), and the pitch of the sounds becomes lower as their intensity greatly increases (cf. sounds 11 to 19). Maximum intensity, as indicated by amplitude of vibrations, was attained in sound number 19, and this sound displays a first vibration of unusually long period, maximum average period, and maximum duration. The number of vibrations per sound reaches its maximum considerably in advance of the greatest intensity of sound (cf. sound 8). As decompression proceeds into the lower levels, intensity diminishes gradually, whereas pitch, after rising slightly, remains practically constant (cf. sounds 20 to 30). When the level of minimum diastolic blood pressure is reached, intensity falls rather abruptly, pitch becomes lower, and the number of vibrations per sound is reduced (cf. sounds 31 and 32). These sounds are comparable in period and intensity with the spontaneous arterial sounds which occur in patients with aortic regurgitation (cf. table 1, C1XO6). The loudest sounds invariably comprise 6 double vibrations of relatively long period, in consequence of which their pitch is comparatively low, and their duration maximal. However, sounds of 5 double vibrations are frequently more intense than sounds which comprise 6 double vibrations (compare sounds 8 and 25), and furthermore, low pitch is not a prerequisite of intensity, for a relatively short average period often exists in conjunction with more than average intensity (cf. sound number 29). My observations relating to vibration frequency are not in agreement with those of Erlanger

TABLE 1

EXPERIMENT NUMBER	NUMBER VIBRATIONS	RANGE IN PERIOD						RANGE IN DURATION	OBSERVATIONS		CARDIOVASCULAR STATUS
		First vibration	Second vibration	Third vibration	Fourth vibration	Fifth vibration	Sixth vibration		Number made	Number above 0.025	
		seconds	*seconds*	*seconds*	*seconds*	*seconds*	*seconds*	*seconds*			
A601	4	0.005–0.007	0.014–0.016	0.016–0.017	0.016–0.019			0.053–0.057	16	0	Normal
A701	3–5	0.014–0.016	0.015–0.016	0.015–0.018	0.015–0.016	0.016–		0.049–0.077	25	0	Normal
A702	5–6	0.012–0.014	0.014–0.016	0.016–	0.015–0.015	0.014–0.016	0.016–	0.072–0.091	32	0	Normal
A703	3–4	0.008–0.015	0.016–0.018	0.014–0.018	0.018–			0.046–0.060	13	0	Normal
A801	3–5	0.009–0.017	0.015–0.019	0.014–0.018	0.016–0.019	0.017–		0.047–0.082	15	0	Normal
A802	3	0.010–0.015	0.017–0.018	0.015–0.016				0.043–0.049	12	0	Normal
A901	4	0.005–0.019	0.012–0.026	0.023–0.030	0.021–0.025			0.068–0.090	16	4	Normal
A902	3	0.021–	0.024–	0.027–				0.072–	6	2	Normal
A1001	3–4	0.010–0.021	0.020–0.021	0.020–0.022	0.022–			0.064–0.080	23	0	Normal
A1102	3–5	0.013–0.020	0.018–0.020	0.018–0.019	0.017–0.020	0.017–		0.056–0.085	16	0	Normal
A1201	5	0.014–	0.018–0.019	0.021–	0.022–	0.021–		0.096–0.097	10	0	Normal
A1306	5–6	0.007–0.014	0.013–0.020	0.020–0.022	0.020–0.022	0.019–0.021	0.020–0.021	0.092–0.111	34	0	Normal
A1408	3–6	0.005–0.015	0.004–0.021	0.018–0.023	0.020–0.024	0.018–0.022	0.021–	0.053–0.118	26	0	Normal
A1510	3–4	0.008–0.021	0.022–0.026	0.023–0.029	0.025–0.027			0.057–0.094	20	6	Normal
A1611	4–6	0.011–0.021	0.015–0.019	0.019–0.020	0.017–0.021	0.019–0.022	0.022–	0.077–0.106	35	0	Normal
A2004	4–5	0.018–0.021	0.019–0.020	0.019–0.020	0.021–0.022	0.021–		0.076–0.103	17	0	Normal
A2107	3–5	0.006–0.026	0.015–0.022	0.019–0.025	0.020–0.026	0.021–0.024		0.061–0.111	37	1	Normal
A2202	5–6	0.006–0.019	0.011–0.022	0.018–0.024	0.021–0.024	0.020–0.024	0.021–0.023	0.072–0.127	172	0	Normal
A2303	5–6	0.005–0.015	0.005–0.018	0.014–0.019	0.018–0.020	0.017–0.020	0.018–0.020	0.070–0.110	39	0	Normal
A2304	5–6	0.004–0.019	0.005–0.021	0.017–0.021	0.019–0.022	0.019–0.027	0.019–0.030	0.095–0.114	51	2	Normal
A2406	3–4	0.016–0.023	0.020–0.021	0.019–0.022	0.021–0.022			0.064–0.085	14	0	Normal

A2408	3	0.019–0.020	0.025–0.026	0.024–0.026				0.068–0.072	6	2	Normal
A2409	4–5	0.013–0.022	0.019–0.020	0.020–0.021	0.020–0.021	0.023–		0.084–0.096	9	0	Normal
A2503	5	0.015–0.016	0.020–0.021	0.019–0.021	0.021–0.022	0.023–0.024		0.100–0.104	20	0	Normal
A2504	4–5	0.015–0.022	0.021–0.022	0.020–0.023	0.021–0.023	0.023–0.024		0.087–0.115	18	0	Normal
A2606	4–5	0.006–0.022	0.016–0.024	0.021–0.024	0.022–0.024	0.022–		0.088–0.017	21	0	Normal
A2803	3	0.009–	0.016–	0.017–0.018				0.042–0.043	6	0	Normal
A2804	3–6	0.008–0.019	0.012–0.024	0.017–0.025	0.020–0.024	0.024–	0.023–	0.046–0.106	27	0	Normal
A2906	3–4	0.006–0.018	0.015–0.017	0.017–0.020	0.018–0.020			0.052–0.061	21	0	Normal
A1X01	6	0.013–0.015	0.017–0.018	0.012–	0.016–	0.013–	0.014–	0.089–	12	0	Normal
A3X02	3–5	0.006–0.018	0.011–0.019	0.016–0.020	0.017–0.021	0.016–0.023		0.047–0.079	50	0	Normal
A3X03	3–5	0.010–0.011	0.014–0.015	0.014–0.016	0.014–0.016	0.014–0 015		0.042–0.069	30	0	Normal
A4X01	3–4	0.005–0.014	0.010–0.013	0.011–0.013	0.012–0.013			0.041–0.050	35	0	Normal
C1X04	4	0.006–0.016	0.012–0.020	0.017–0.028	0.010–0.028			0.051–0.078	20	1	Aortic regurgitation
C1X05	3	0.014–	0.017–	0.017–				0.048–0.049	6	0	Aortic regurgitation
C1X06	2	0.024–0.025	0.023–0.025					0.048–0.050	4	0	Aortic regurgitation
C2X06	3–6	0.008–0.028	0.008–0.020	0.015–0.021	0.015–0.026	0.017–0.019	0.018–	0.066–0.091	40	3	Aortic regurgitation
C2X07	2–3	0.012–0.026	0.013–0.019	0.016–0.018				0.041–0.061	16	3	Aortic regurgitation
C501	3	0.016–0.023	0.020–0.021	0.018–0.021				0.057–0.064	18	0	Aortic regurgitation
Average period..		0.0144	0.0181	0.0192	0.0196	0.0197	0.0193	Totals...	988	24	
Average frequency.....		69	55	52	51	50	51				

TABLE 2

*Experiment A2202**

SOUND NUMBER	SOUND										PULSE				
	Intensity (maximum amplitude of vibrations)	Vibration periods							Average frequency (dv per second)	Duration	Column 1	Column 2	Column 3	Column 4	Column 5
		First	Second	Third	Fourth	Fifth	Sixth	Average			Time between beginning of pulse and sound	Time between sound and first crest of pulse	Time from beginning of pulse to first crest	Amplitude of pulse to first crest	Amplitude of pulse between sound and first crest
	mm.	*seconds*	*seconds*	*seconds*	*seconds*	*seconds*	*seconds*	*seconds*		*seconds*	*seconds*	*seconds*	*seconds*	*mm.*	*mm.*
1	12.0	0.010	0.017	0.022	0.023			0.018	55	0.072					
2	11.0	0.010	0.016	0.022	0.021	0.023		0.018	55	0.091					
3	10.0	0.009	0.016	0.021	0.021	0.022		0.018	55	0.091					
4	9.5	0.009	0.016	0.022	0.023	0.022		0.018	55	0.092					
5	9.0	0.009	0.016	0.022	0.023	0.024		0.018	55	0.093					
6	7.0	0.008	0.016	0.020	0.023	0.023		0.018	55	0.091	0.091	0.027	0.118	14.5	3.5
7	8.0	0.008	0.015	0.021	0.022	0.022		0.017	58	0.088	0.086			14.5	
8	9.0	0.006	0.012	0.018	0.022	0.022	0.022	0.017	58	0.104					
9	11.0	0.006	0.011	0.019	0.022	0.023	0.022	0.017	58	0.103					
10	12.0	0.006	0.012	0.019	0.023	0.023	0.022	0.017	58	0.105					
11	13.0	0.009	0.015	0.022	0.023	0.023	0.021	0.019	52	0.113					
12	13.0	0.009	0.015	0.020	0.023	0.022	0.022	0.019	52	0.113					
13	13.0	0.011	0.016	0.021	0.024	0.022	0.023	0.020	50	0.117					
14	12.0	0.015	0.019	0.021	0.024	0.023	0.022	0.021	47	0.124					
15	14.0	0.013	0.017	0.021	0.023	0.022	0.023	0.020	50	0.120	0.066	0.034	0.100	14.0	5.8
16	14.0	0.013	0.017	0.021	0.023	0.022	0.022	0.020	50	0.118	0.057			14.0	
17	15.0	0.015	0.020	0.023	0.023	0.021	0.023	0.021	47	0.125					
18	17.0	0.017	0.020	0.022	0.022	0.023	0.023	0.021	47	0.127					
19	18.0	0.019	0.020	0.022	0.022	0.022	0.023	0.021	47	0.127					

20	16.0	0.015	0.019	0.022	0.021	0.022	0.023	0.020	50	0.122					
21	14.5	0.015	0.020	0.023	0.022	0.021		0.020	50	0.100					
22	14.5	0.015	0.020	0.023	0.022	0.022		0.020	50	0.102					
23	13.5	0.015	0.019	0.023	0.021	0.020		0.020	50	0.098					
24	14.0	0.014	0.020	0.023	0.021	0.021		0.020	50	0.099					
25	15.0	0.014	0.019	0.023	0.021	0.021		0.020	50	0.099					
26	14.0	0.015	0.020	0.023	0.021	0.021		0.020	50	0.100	0.039	0.026	0.065	9.0	3.4
27	14.0	0.015	0.020	0.024	0.021	0.021		0.020	50	0.100	0.039			8.0	
28	14.0	0.015	0.019	0.024	0.021	0.021		0.020	50	0.100					
29	14.0	0.014	0.019	0.023	0.021	0.020		0.019	52	0.097					
30	13.0	0.013	0.020	0.023	0.021	0.021		0.020	50	0.098					
31	9.0	0.014	0.019	0.023	0.022	0.021		0.020	50	0.098					
32	8.0	0.017	0.022	0.023	0.021			0.021	47	0.083					
Average period..		0.0123	0.0175	0.0218	0.0220	0.0219	0.0225								
Average frequency.....		81	57	46	45	46	44								

* Blood pressure 120–75. Allowing for interruptions, the average rate of deflation was approximately 2 mm. Hg per beat.

(1916), who reports a frequency of approximately 172 dv per second for the third phase sounds, and 133 dv per second for the first and early fourth phase sounds. Discussion of this disparity will be deferred.

No constant differences in number of vibrations, period, duration, or intensity can be detected between the sounds obtained with the Erlanger cuff and those with the ordinary commercial cuff, but when an unusually narrow cuff, measuring 6 cm. in width, is employed, significant changes appear in the recorded sounds. Periods remain essentially the same, but the number of vibrations per sound, the duration, and the intensity are considerably reduced, and the total number of sounds recorded becomes much less. Furthermore, the narrow cuff accentuates murmurs. Very short, faint murmurs were not satisfactorily registered, but when frankly audible murmurs occurred the capsule recorded them. An example of such a murmur, obtained with the wide cuff, is reproduced in figure 1F. Figure 1G was taken from the same subject as figure 1F, but with the narrow cuff substituted for the wide cuff. A considerable increase in the magnitude of the murmur is apparent. Hooker and Southworth (1914) made use of a cuff 5 cm. wide in some of their experiments, but they make no mention of murmurs, nor are murmurs discernible in their published records.

Reduplication of the arterial sounds is an interesting phenomenon. In some subjects the sounds are distinctly double at certain pressure levels, as if two hammers were striking barely asynchronously. This was particularly well exemplified in one of my subjects, a patient with aortic regurgitation. The first five or six sounds to appear were clearly double, after which the reduplication was abruptly lost. In three separate experiments with this patient a striking change in the character of the recorded sounds took place, corresponding in time, and apparently in quality, with the auscultatory findings. One of these records is reproduced in figure 2. It should be noted that the first five sounds, which were audibly reduplicated, are distinguished by two or three main vibrations of practically equal amplitude, the second occasionally larger than the first. These are abruptly succeeded by sounds, of which numbers 6 and 7 are examples, whose appearance is totally different by reason of the fact that they contain only one main vibration of considerable amplitude. The explanation which immediately suggests itself is, that the sound-recording membrane, receiving in rapid succession two identical sounds, the second coming at the instant when oscillation from receipt of the first is at its maximum, responds simply by additional main vibrations, giving the picture of a single sound characterized by unusual uniformity and duration of its main vibrations. In other words, twin sounds which the ear interprets as barely incoincident are of such a contour in the records that upon simple inspection of them one would immediately postulate a sound of "dull" quality, in

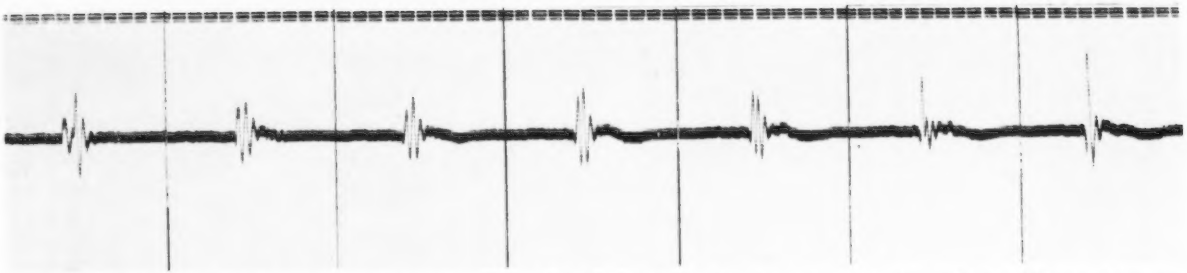


Fig. 2. Seven successive sounds from one subject, showing abrupt change in character beginning with the 6th sound, corresponding with audible transition from a reduplicated to a single sound. ($\frac{7}{10}$ actual size.) Time 0.04 second.

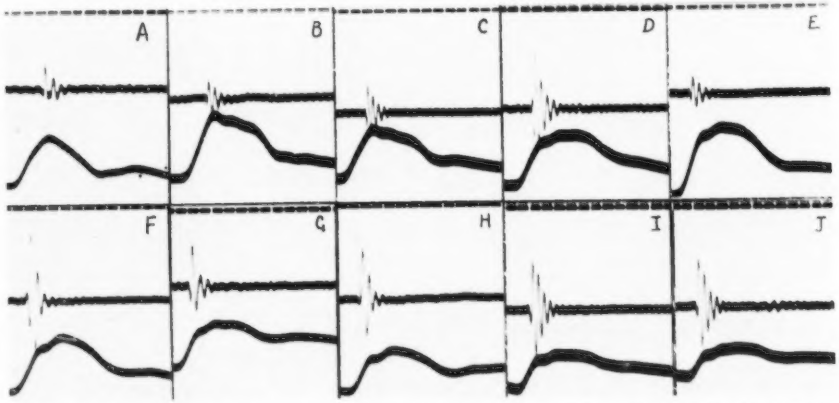


Fig. 3. Ten segments of compression pulse records, showing qualitatively the change in form which the pulse undergoes during progressive decompression from the point of maximum systolic, to the point of minimum diastolic blood pressure. ($\frac{1}{2}$ actual size.) Time 0.04 second.

decided contrast to the succeeding sounds of "sharp" quality. In view of the small number of observations, this explanation is offered only as a tentative suggestion.

The Korotkov sounds in patients with aortic regurgitation differ from normals only in intensity. In all other respects they are strictly comparable (cf. table 1).

Relation of the sounds to the compression pulse. During decompression from the level of maximum systolic, to that of minimum diastolic pressure, the compression pulse in man undergoes a progressive and constant change in form. This is illustrated by figure 3.[2] At first monocrotic, with a rather sharp crest (segment A), it quickly develops two crests and a broad top (segments B, C, D). Its amplitude, measured to the highest summit, increases at first, but later falls to a value much below the initial amplitude. The amplitude of the first crest, however, remains roughly constant until the middle pressure levels are reached, when it begins to decline in proportion to the fall of total amplitude (cf. table 2, column 4). Inasmuch as the amount of time which elapses between the beginning of the upstroke and the top of the first crest progressively decreases from beginning to end (cf. table 2, column 3), it is obvious that the gradient of the upstroke grows progressively steeper at least until the amplitude of the first pulse crest begins to decline.

The time interval which elapses between the beginning of the upstroke of the pulse and the beginning of the sound progressively decreases (cf. table 2, column 1, and fig. 3A, D, F), and this decrease is a direct measure throughout decompression of the relation of the sounds to the compression pulse. In the higher levels of decompressing pressure this decrease is partly the result of increasing steepness in the gradient of the ascending limb of the pulse, but that this is not the most important factor is proved by the fact that at the same time there is a proportionately greater increase not only in the amount of time which elapses between the incidence of the sound and the first crest of the pulse (cf. table 2, column 2), but also in that part of the total pulse amplitude which is included between these two points (cf. table 2, column 5). If we bear in mind that the amplitude of the pulse to the first crest remains practically unchanged throughout this period, it is at once apparent that the sounds actually begin earlier with respect to the pulse. The sounds move down the ascending limb of the pulse, so to speak (cf. fig. 3A, B, C,D). Figure 4 serves to illuminate some of the points involved in this analysis of pulse-sound relationship. In this figure, *c* marks the first crest of the pulse, and *s* indicates the point on the

[2] The individual segments of this figure are not all taken from the same experiment, and therefore are not comparable quantitatively. A record of every pulse in each experiment would be desirable, but it is obviously impossible to obtain such a record with a method which requires a closed system for pulse registration.

ascending limb which is synchronous with the incidence of the sound. Therefore, *sa* represents the time interval which elapses between the incidence of the sound and the first crest of the pulse, and *ca* represents that part of the total pulse amplitude which is included in the interval between the beginning of the sound and the first crest of the pulse. If *s'* be taken as one of many succeeding positions of the sound in relation to the pulse, it is obvious that the values represented by *sa* and *ca* become proportionately increased to *s'a'* and *ca'*, respectively. In the lower pressure levels, although both the time interval between the incidence of sound and first pulse crest (fig. 4, *sa*), and the amplitude of the pulse between these two points (fig. 4, *ca*) are decreasing (cf. table 2, columns 2 and 5), so that the sound apparently moves back toward the crest, it will be found that the amplitude of the first pulse crest, as well as the time consumed in attaining it, is now rapidly declining (cf. table 2, columns 3 and 4), so that in reality the crest is coming down toward the sound while the latter continues its steady descent along the ascending limb of the pulse (cf. fig. 3G, H, I, J).

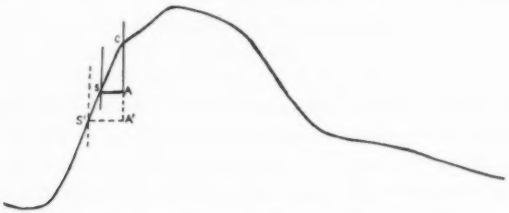


Fig. 4. Diagrammatic drawing of a compression pulse curve, introduced to clarify some of the points involved in the analysis of pulse-sound relationship. See text.

When pressure within the cuff is above the level of maximum systolic blood pressure, sudden and complete arrest of the arterial pulse against the upper margin of the cuff produces fluctuations of pneumatic pressure which are recorded by the sensitive optical capsule in the form of a monocrotic curve with rather sharp crest. When pneumatic pressure falls just below the level of maximum systolic blood pressure the fundamental mechanism responsible for the compression pulse remains essentially unchanged, except that the small jet of blood thereby permitted to penetrate the artery beneath the cuff at topmost arterial pressure levels augments the amplitude of the curve to some extent. Penetration by the jet sets into play the mechanism which produces the Korotkov sounds, and inasmuch as penetration is at first restricted to the highest systolic pressure levels we shall find that the sounds have their beginning but an instant before the compression pulse curve attains its peak (cf. fig. 3A).

As decompression proceeds, increasing volume of the penetrating jet opens the compressed artery farther, more rapidly, and at lower arterial

pressure levels, with the result that the contour of the compression pulse becomes more the expression of volume and pressure fluctuations within the artery beneath the cuff. The gradient of the ascending limb grows steeper, and a second crest makes its appearance. The first crest of the pulse may be regarded as the termination of water hammer, and the second as the result of volume changes within the compressed artery (Erlanger, 1916). At the higher pressure levels, in which the force of water hammer overshadows volume changes produced by the penetrating jet, the first crest stands at a higher level than the second (cf. fig. 3A, B, C). At lower pressures, although there is a contemporaneous increase in the force of water hammer, volume changes reach a magnitude which elevates the second crest to a level higher than the first, from which position it is not subsequently displaced (cf. fig. 3D, E, F, G, H, I, J). Bearing in mind the fact that penetration by the jet and incidence of the compression sound are synchronous events, penetration at successively lower arterial pressure levels determines progressively earlier incidence of the sounds with respect to the initial rise of the compression pulse.

Discussion. The mechanism of the compression sounds of Korotkov has been the subject of much speculation. Without recounting all the explanations which have been put forward, it may be said that a sufficiently convincing solution of the problem has been offered by Erlanger (1916), who demonstrated in animal experiments that water hammer action is alone responsible for the sounds. Although the question is not susceptible of direct proof, there is every reason to assume that the same mechanism operates to produce arterial sounds in man. It is pertinent, therefore, to canvass the data of human experimentation for evidence which might tend to support or to disprove the water hammer hypothesis.

In analyzing the compression sounds I have called attention to several discrepancies between my results and those of Erlanger. The first concerns introductory vibrations of short period and small amplitude, which are most numerous in the early sounds, occasionally present in late sounds, but conspicuously wanting in the loud sounds at middle compression levels. In Erlanger's records a small initial vibration of very short period appears only when the sounds are most intense. He designates this a "preliminary" vibration, and assumes that it is produced "by sound transmitted by the blood stream as such, from the point where sound first originates, and that the powerful vibrations represent sound waves started locally within the stethoscope by the arrival there of a transmitted impact." Such an assumption in itself seems hardly justifiable, and is unnecessary in the light of a more sensitive method which discovers no association between sound intensity and occurrence of introductory vibrations. On the contrary, these vibrations must be regarded as the actual beginning of the sound, but this fact does not endanger the water hammer hypothesis.

The second discrepancy between my results and Erlanger's is found by comparing the observations relating to vibration frequency, which discloses the fact that his vibration rates are approximately three times as rapid as mine. It is possible that such a great difference in the pitch of the arterial sounds of the dog and man actually exists, but in order to settle the question it would be necessary to repeat the animal experiments with more sensitive sound-registration apparatus than Erlanger employed. In either event, the water hammer theory suffers no modification.

The third point of disagreement concerns the mode of onset of the individual sounds. Erlanger reports that the first main vibration of each sound is always the highest, and, in view of the fact that the initial force of the impact produced by water hammer is theoretically high, he is led to postulate a water hammer sound of maximum initial intensity. He interprets its occurrence, therefore, as evidence in favor of the hypothesis. The small "preliminary" vibrations have already been discounted, and are not taken into consideration in this connection. As I have indicated, many of the Korotkov sounds in my records display a distinctly non-abrupt onset which is determined either by an initial *main* vibration of submaximal amplitude, or by the occurrence of *introductory* vibrations which must be recognized as the actual beginning of the sound. Instead of modifying the water hammer hypothesis, however, these contradictory findings may lead to a better understanding of the hydraulics of water hammer at different levels of decompressing pressure. If the hydraulic impact of water hammer is always characterized by maximum initial force, as Erlanger has suggested, and if initiation of the impact is reflected in the initial vibrations of the sound, why is it that *introductory* vibrations frequently initiate the sounds at high and low pressure levels, whereas a *main* vibration invariably initiates the sounds at middle pressure levels? The ratio between the initial and final force exerted by the hydraulic impact of water hammer varies in inverse proportion to total water hammer intensity. When water hammer intensity increases, the ratio falls as a direct result of reduction in the initial force of the impact, the final force remaining practically unchanged. The higher the ratio, the higher the frequency of the initial sound vibrations. Consequently, at high and low pressure levels, when water hammer intensity is submaximal, there is a relatively wide range in frequency between the initial and final vibrations, extending in the extreme case from 256 to 40 dv per second; and at middle pressure levels, when water hammer intensity is maximal, the frequency of initial vibrations does not exceed 100 dv per second, whereas that of the final vibrations remains at 40 dv per second. It becomes apparent, therefore, that initiation of the hydraulic impact is essentially related, not to intensity of sound, but to pitch, for although the amplitude of the first sound vibration may vary from one extreme to the other, its frequency is always high relative to that of succeeding vibrations. Inasmuch as the

same conclusion might logically be reached from theoretical consideration of the nature of hydraulic impact, this new conception is offered in support, rather than in disproof, of the water hammer hypothesis.

The question of where the Korotkov sounds originate has been answered satisfactorily by Erlanger (1916). Further evidence in support of the idea that the sounds arise uniformly and solely from the receipt of an impact at one fixed spot is furnished in my records by the general similarity of the vibrations at all levels of decompressing pressure.

My analysis of the relation of the arterial sounds to the compression pulse is open to certain criticisms. It is well known that the method which I employed for pulse registration is inadequate. A pneumatic system of large volume under varying pressure produces considerable distortion of the curve (cf. Wiggers, 1923). However, no other method applicable to man is available. It is necessary, therefore, to compare my results with those obtained in animal experiments by Erlanger (1916). Such a comparison reveals good qualitative agreement in every detail wherein disagreement might necessitate revision of the conclusions already reached, but at one point yet to be considered an appreciable disparity is discovered. Erlanger finds that the intensity of the recorded sounds corresponds with the intensity of water hammer, and that water hammer intensity may be measured roughly by the ratio of amplitude of that part of the compression pulse subtended by the marks of onset of sound and first crest (fig. 4, *ca*), to the time elapsing in that interval (fig. 4, *sa*). My figures do not agree with Erlanger's, inasmuch as the most intense sounds in my records always appear either a little after the peak of this ratio, or continue at maximum intensity for some time after the peak is attained. They are probably unreliable, therefore, in so far as determination of water hammer intensity is concerned.

Most of the variations in the quality of the Korotkov sounds, such as reduplication, murmurs, and changes in intensity and duration, are readily recognized clinically, but so far as I am aware, there has been no expression of opinion concerning pitch. Such opinions would be of little value, however, for in the presence of great differences in intensity, the ear cannot be relied upon to distinguish small differences in pitch. No reliable information can be gained except by a purely objective physical method. By means of such a method it is found that in general the pitch of the Korotkov sounds is slightly higher in the upper levels than it is in the middle and lower levels of decompressing pressure.

It is of interest to consider the association of murmurs with the Korotkov sounds. Murmurs are usually described as occurring in one or another of the so-called "phases" of sound which are supposed to succeed one another during decompression. However, it has been my experience that when a cuff of the proper width is employed, frank murmurs of appreciable magnitude and duration are rarely encountered. The sounds sometimes ac-

quire a somewhat murmurish character at certain pressure levels, but even this is far from being the common event which one is lead to expect. Endovascular murmurs arise from the eddies which are produced when a blood stream flowing with sufficient velocity emerges suddenly from a relatively small into a relatively large lumen. Partial occlusion of an artery at one point is enough to produce a murmur, but the pistol shot sound is conspicuously wanting. This pistol shot sound depends upon water hammer, and water hammer is dependent upon extremely wide variations of endovascular pressure. The loud water hammer sounds which occur spontaneously in the peripheral arteries of patients with aortic regurgitation are never accompanied by murmurs. Furthermore, I have shown that the employment of a cuff which is admittedly too narrow to be capable of developing the full action of water hammer interferes with the normal character of the Korotkov sounds about to the same extent that it promotes the formation of murmurs. From these facts it may reasonably be inferred that the conditions which are essential to water hammer are unfavorable for the formation of eddies in the blood stream, and that partial occlusion of an artery at one point does not bring water hammer action into play.

In conclusion, a word should be said concerning the five classical "phases" of the Korotkov sounds. Careful observation of many compression sound sequences, both from the subjective and objective standpoints, leads to the opinion that this division into "phases" is unnaturally dogmatic and unworthy of the emphasis which has been placed upon it. It is the exception rather than the rule to find sound sequences which display the supposedly characteristic "phase" differentiation. A much more generally applicable statement would be that, during decompression from the point of maximum systolic pressure to the point of minimum diastolic pressure, the sounds begin sometimes softly, sometimes sharply, and continue with varying quality and intensity, often becoming most intense in the lower pressure levels, until, with more or less abruptness, they disappear, or become very faint.

SUMMARY

1. In the normal man, and in patients with aortic regurgitation, the Korotkov compression sounds are composed of a series of regular vibrations of generically uniform character. The number of vibrations per sound varies from three to six. The vibration periods range from 0.0039 to 0.025 second (e.g., between 256 and 40 dv per second), and the duration varies from 0.041 second in a sound of four double vibrations to 0.1275 second in a sound of six double vibrations. The duration of a sound depends not only upon the number of component vibrations, but also upon the duration of component vibration periods. The period of the first vibration is always shorter than that of succeeding vibrations. On the basis of certain characteristic differences in period and amplitude the vibra-

tions are divided into three groups which are designated as *introductory*, *main*, and *final*. The first *main* vibration is usually the one of greatest amplitude, but in some sounds the second is higher than the first, and in others the main vibrations are all of nearly equal amplitude. The loudest sounds invariably comprise 6 double vibrations of relatively long period, in consequence of which their pitch is comparatively low, and their duration maximal. However, sounds of 5 double vibrations are frequently more intense than sounds which comprise 6 double vibrations, and furthermore, low pitch is not a prerequisite of intensity, for a relatively short average period often exists in conjunction with more than average intensity. During progressive decompression the pitch of the sounds shows a general tendency to fall slightly as their intensity increases. The employment of an unusually narrow cuff interferes seriously with the normal character of the sounds, but promotes the formation of murmurs. Frank murmurs of appreciable magnitude and duration rarely occur when a cuff of the proper width is employed. Reduplication of the arterial sounds is an interesting phenomenon occasionally encountered.

2. The changes occurring in the form of the compression pulse during decompression are analyzed, and it is demonstrated that penetration of the compressed artery at successively lower arterial pressure levels determines progressively earlier incidence of the Korotkov sounds with respect to the initial rise of the compression pulse.

3. Contributory evidence is brought forward in support of the reasonable assumption that the mechanism which operates to produce the Korotkov sounds in man is identical with the water hammer action which Erlanger has found to be the cause of arterial sounds in animals. Furthermore, it is demonstrated that initiation of the hydraulic impact of water hammer is characterized by sound vibrations of relatively short period.

4. The general similarity of the vibrations at all levels of decompressing pressure indicates that the Korotkov sounds originate in an impact at one fixed spot. There is no evidence that eddies or whorls contribute to sound production.

5. Division of the Korotkov sounds into so-called "phases" seems unwarranted. It is suggested that in this respect our clinical conception of the sounds is in need of revision.

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EGG YOLK AS A SOURCE OF THE ANTI-SCORBUTIC VITAMIN

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Received for publication December 19, 1925

That the hen's egg is lacking in the anti-scorbutic vitamin was pointed out in 1918 by Hess and Unger (1) and more recently after the author's work was completed by Hauge and Carrick (2). The earlier workers fed the entire contents of fresh eggs without separating the yolk from the white. In the work reported in this paper the yolk and white were fed separately as was done by Hauge and Carrick and for the ten-day period previous to the beginning of the experiment, the guinea pigs used were fed all the fresh, green herbage they would consume so that when they were placed on the experimental diet, their tissues would be stocked with a maximum amount of the anti-scorbutic vitamin.

EXPERIMENTAL. Young guinea pigs weighing from 325 to 510 grams and averaging 430 grams each were divided into 14 groups of two each and placed in separate cages on wire screen bottoms in the laboratory animal room.

The basal scorbutic ration was fed ad libitum and consisted of 69 per cent of finely ground, steam rolled, hulled oats, 25 per cent alfalfa meal, 5 per cent crude casein and 1 per cent NaCl. Each group of animals also received 2 cc., 4 cc. or 8 cc. per animal daily of either fresh egg yolk or egg white obtained from eggs laid in November and December from flocks of yarded fowls well supplied with fresh greens. This material was administered per os by means of a 10 cc. glass syringe to the delivery tube of which was attached a $1\frac{1}{4}$ inch piece of fine rubber tubing. The tubing was found to be very helpful in delivering the egg yolk or white well back in the mouth and insuring the swallowing of the full dose without waste.

The egg substance was administered to the animals as follows:

Groups 1 and 2—2 cc. of fresh egg white per pig daily
Groups 3 and 4—4 cc. of fresh egg white per pig daily
Groups 5 and 6—8 cc. of fresh egg white per pig daily
Groups 7 and 8—2 cc. of fresh egg yolk per pig daily
Groups 9 and 10—4 cc. of fresh egg yolk per pig daily
Groups 11 and 12—8 cc. of fresh egg yolk per pig daily
Groups 13 and 14—controls—no egg substance fed

The first death occurred in group 12 (8 cc. yolk) on the 5th day of the experimental period. It was followed within a week by the deaths of the remaining 3 pigs in the groups receiving 8 cc. doses of yolk and of both pigs in group 10 (4 cc. yolk). On the 17th day a pig died in group 7 receiving 2 cc. of yolk.

Post-mortem examination of these pigs revealed no scurvy lesions whatever. The wrists, knees, thighs and costo-chondral junctions were normal, the teeth were not loose and there were no hemorrhagic lesions in the intestinal area. The animals had become quite emaciated, however, and death seemed to be due to the injurious effects of the egg yolk which was apparently difficult for the animals to digest. In this respect the yolk seemed to be more difficult for the animals to handle than the egg white, especially in the larger doses fed.

The first death revealing unmistakable ante-mortem symptoms and pronounced post mortem lesions of scurvy occurred in group 7 (2 cc. yolk) on the 22nd day. The remainder of the animals receiving yolk now began to drop off one by one and were followed by those receiving egg white. In all of these animals the symptoms and lesions of scurvy were very definite.

Due to the number of deaths occurring in the yolk-fed groups in the early stages of the experiment, 3 more groups of two pigs each were started. Two of these groups (15 and 16) were given 3 cc. of egg yolk per pig daily and the other group 4 cc. per pig. The animals in these last 3 groups did not begin to die until the 25th day and all were dead within 3 days. With these animals also the ante-mortem indications such as swollen wrists, soreness of the ribs and disinclination to move or be handled as well as the post-mortem findings definitely indicated scurvy just as they had with the previous groups.

Discussion. In any of the dosages used egg yolk and egg white were not found to possess any anti-scorbutic properties in preventing the onset of scurvy in guinea pigs. Egg white seemed to be able to delay the onset of scurvy as compared with egg yolk, but this may have been due to the fact that the yolk was more difficult to digest and assimilate. Egg yolk was much more distasteful to the animals than egg white and the considerable number of deaths occurring early in the experimental period from among those animals receiving the larger dosages of egg yolk would indicate the inability of guinea pigs to successfully assimilate this substance in any considerable amounts. Hauge and Carrick do not mention this point in their paper although they fed as high as 15 cc. of egg yolk daily.

The increase in weight of the animals receiving egg white continued somewhat longer and the succeeding loss in weight went on somewhat more slowly than with the yolk fed animals.

The author wishes to tender his sincere thanks to Dr. H. Steenbock under whose guidance this work was conducted at the Department of Agricultural Chemistry of the University of Wisconsin.

SUMMARY

Fresh egg white and egg yolk administered in amounts ranging from 2 cc. to 8 cc. per animal daily were not found to be of any value in preventing scurvy in guinea pigs.

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EFFECTS OBTAINED FROM FEEDING FRESH ADRENAL CORTEX, MEDULLA AND WHOLE GLAND TO THE STANDARD WHITE RAT

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Received for publication December 22, 1925

In 1916, R. G. and A. D. Hoskins (1) reported a series of controlled experiments in which white rats were fed desiccated adrenal gland. These animals were fed for varying periods from two to nine weeks after which the weights of the various organs and the rate of growth were determined. It was found that the spleens of the experimental series were somewhat smaller than those of the control animals; that the testes of the experimental animals showed hypertrophy and the ovaries in the few cases studied were larger in the experimental series. These authors concluded that the testicular hypertrophy was probably due to the cortical portion of the gland.

In so far as we have been able to ascertain, all feeding experiments with adrenal gland have been carried on with the desiccated preparation of the whole gland. Since a great deal of significance has been attached to the lipoid substance present in the cortex, it seemed to us that feeding experiments in which desiccated whole gland was fed, purporting to demonstrate the importance of the cortex and more especially its lipoid substances are not conclusive. According to the United States Pharmacopoeia, desiccated suprarenal gland is freed from all fat. Any changes then experienced from feeding desiccated whole gland cannot be attributed to the cortex and much less to the lipoids contained therein. Stewart (2) recently has given an excellent review of the literature on the adrenal gland to which the reader is referred. He emphasized the lack of definite metabolic data on the supposed relations of the cortex to the sex glands and the meaning of the cortical lipoids.

The following experiments were untertaken to determine the effect of feeding adrenal glands which were obtained as nearly fresh as possible, and were begun at Baylor University in the early part of 1924.

METHOD. The standard white rat obtained from the Wistar Institute was used in all the feeding experiments. All were male rats from the same

litter varying in age from 14 to 21 days. The sixty-four rats used were placed in separate wooden cages 8 × 8 × 12 inches. The animals were received in excellent condition, weighed, and were then placed on a standard diet which was maintained throughout the experiment.

The diet used consisted of equal parts of lima beans, whole wheat, rice and rolled oats. These ingredients were first cooked in water with the addition of enough salt to taste, and finally in an autoclave for one hour at 15 pounds pressure. At the beginning of the experiment each rat was given about 15 grams of food each day with as much fresh milk as the food would absorb. Water was given ad libitum. On alternate days a leaf of cabbage was given to each animal. During the course of the experiment the amount of food was increased as the needs of the animals seemed to indicate. The animals were weighed at weekly intervals.

In addition to the above diet, sixteen rats were started with one gram daily of fresh beef adrenal gland (whole gland); sixteen rats were given one gram of fresh beef adrenal cortex for the first week after which the amount of gland was increased to three grams daily for the succeeding eleven weeks; sixteen rats were started with one gram daily of fresh beef adrenal medulla while the remaining sixteen rats were kept as controls. It became necessary in the course of the experiment to make some changes in the above classification. The rats were fed late each afternoon. On Saturdays double portions were given because the animals were not fed on Sundays. Each rat was supplied with two feeding pans so that the amount of the diet and adrenal gland consumed could be easily determined.

Adrenal glands for the experiment were obtained from the Swift Packing Co. of Fort Worth, Texas, twice each week. After slaughtering the cattle, the adrenals were removed and chilled. They were immediately delivered to us, wrapped in oiled paper, packed in ice. In none of the shipments received was there any apparent post-mortem autolysis or decomposition noted. The chilled glands were sectioned through the middle, the cortex removed with scissors, leaving a part of the cortex with the medulla. Some of the whole glands were ground up in a meat grinder and kept at a very low temperature for the whole gland experiments. The medulla with some cortex attached was similarly passed through the meat grinder and kept at a low temperature.

RESULTS. *Feeding of fresh whole gland and medulla.* After a few days' observation on the standard diet rats 9 to 16 and rats 58 to 64 were given one-gram portions of the whole gland in addition to the regular diet. Rats 17 to 32 were given one-gram portions of fresh medulla (small amount of cortex attached). We were surprised the following morning to find that rats 11, 59, 60 and 62, all of which had been given whole gland, were dead. Rats 21, 22 and 27 which were fed on medulla were also dead. Rats 43 to 57 had been given one gram of fresh adrenal cortex with no apparent

effect. Some of the other rats in the whole gland series and medulla series appeared very depressed, some of them having a severe diarrhea. It occurred to us that we were probably feeding too much gland so the next day the amount was reduced to one-half gram. Again on the following morning rats 10, 11 (another rat had been substituted for this number from stock), 14, 15 and 64 were found dead. All had received one-half gram of fresh whole adrenal gland. In the fresh medulla series rats 23 and 24 were found dead. The same day rat 50 of the cortex series died. Since this was the only death in the cortex series throughout the entire period of the experiment, and the other animals were apparently normal, we were at a loss to explain this death, unless we admit the possibility of there having been some medulla tissue left attached to the fragment of cortex this animal consumed which might have been overlooked in cutting the cortex away from the gland.

During the first week of the experiment we had experienced fifteen deaths from the feeding of fresh adrenal gland. Fourteen of these deaths occurred in those rats receiving whole gland or medulla. Only one rat in the cortex series had died and the other members of this group were apparently unaffected. The fact that the animals were young and for that reason were more easily affected by the fresh whole gland and medulla suggested itself to us. Accordingly we decided to discontinue the feeding of whole gland and medulla for several days. The animals were weighed again on the fourth day of the experiment. It was found that the control rats had made a uniform gain of from 6 to 13 grams, or an average of 11.1 grams during the first four days. Rats 9 to 32 inclusive showed a gross loss in weight of 51 grams, ranging between a loss of 9 grams to a gain of 3 grams when the individuals were considered (tables 1 and 2).

At the end of the first week of the experiment the animals were again weighed. This was three days after discontinuing the feeding of fresh whole gland and medulla. The control animals made an average gain during this three-day period of 4.6 grams. Rats 9 to 32 gained from 1 to 10 grams, or an average of 7 grams.

On the tenth day of the experiment, or seven days after the fresh whole gland and medulla had been discontinued, rats 9 to 16 were again given one-half gram amounts of fresh whole gland and rats 17 to 28 were given one-half gram amounts of fresh adrenal medulla. None of the animals would eat it. Weights taken at this time (3/8/24) showed a steady gain in all. The control aimals showed a gain of from 13 to 18 grams, or an average of 12.7 grams. Rats 9 to 28 showed a gain of from 6 to 19 grams or an average of nearly 14 grams. During the following week (3/8/24 to 3/15/24) animals 9 to 28 were given one gram portions daily of the fresh whole gland which, in so far as we could determine, remained untouched. Weights taken at the end of this period showed a gain of from

one to eighteen grams. There was an average gain in the controls for this week of 9.2 grams, and the average gain in the feeding series was 8.75 grams. The same procedure was followed during the next week. At the end of this period weights were taken (3/22/24) and showed an average gain in the control animals of 8.37 grams while the feeding series showed an average gain of 8.75 grams. Some new method of feeding had to be used in order to get the rats to eat the fresh gland. On 3/23/24 one gram portions of the whole gland and medulla were mixed with the diet, and on 3/25/24 rats 19, 22, 23 and 25 were found dead. By this time the rats weighed more than twice their weight when the experiments were begun.

It was apparent from the above experiments that fresh whole adrenal gland or the fresh medulla when eaten by the rats in quantities somewhat under one gram, as none of the animals consumed the entire amount of gland given to them, was toxic and would produce rapid death.

The first possible explanation which suggested itself lay in the adrenalin content of the gland, particularly the medulla of the gland. We wondered, however, if other animals were affected in the same way when fresh gland was fed. Young pups weighing around 2000 grams were given one or two fresh whole beef adrenal glands and it was noted that in from 30 to 45 minutes the animals would vomit the material, showing that they were definitely nauseated. After they had vomited the glandular material they again appeared normal. Whether these animals would have died had they been unable to vomit, as is the rat, we cannot say. However, the experiment shows that even in dogs fresh adrenal gland when taken by mouth produces untoward effects.

Feeding of a 1:1000 solution of adrenalin chloride to rats. Adrenalin is supposed to have little effect when administered by mouth to normal animals, although Leyton states that elevation of blood pressure is brought about in cases of Addison's disease when adrenalin is administered by this route. Aschner and Pisk (3) also have recently reported an increase of blood pressure following the ingestion of from 3 to 4 cc. of a 0.1 per cent solution of epinephrin, though they state that the test frequently produces pain and vomiting. It seemed desirable to determine the effects of adrenalin in rats when administered by mouth. On 3/27/24, rats 24, 26, 27, and 28 weighed 69, 80, 70 and 71 grams respectively. These animals were given 2, 2, 2 and 4 cc. of a 1:1000 solution of adrenalin chloride (Parke-Davis & Co.) in a small amount of the standard diet. The food was sufficiently dry to absorb these quantities. The animals, having been previously starved, ate all of the food and adrenalin immediately with no demonstrable symptoms. Later one rat was given 10 cc. of this solution in the same manner without effect. One cubic centimeter of this solution injected subcutaneously in a control animal weighing 70 grams produced convulsions, profuse bleeding from the nostrils and death within seven minutes.

TABLE 1

	RAT	2/26	3/1	3/4	3/8	3/15	3/23	3/26	4/2	4/9	4/16	4/23	5/1	5/3	5/10	5/13	5/17
Controls	1	36	42	50	63	70	79	89	108	102	121	123	157	×	151	162	177
	2	32	43	48	64	71	79	88	105	106	126	122	159	×	172	176	188
	3	35	47	51	65	78	86	96	116	116	130	125	157	×	172	177	181
	4	34	45	51	65	77	84	97	110	116	130	130	156	×	167	178	189
	5	32	44	49	67	71	80	94	107	110	124	127	155	×	166	172	178
	6	39	52	55	68	78	86	100	120	117	123	129	159	×	172	177	183
	7	38	49	51	66	77	86	96	111	115	127	127	155	×	176	177	179
	8	33	46	50	65	75	85	98	115	119	130	122	154	×	170	175	180
Received whole gland	9	36	36	44	55	73	77	89	110	109	117	112	140	×	×	×	×
	10	**43**,41	41	51	66	72	75	84	**96**	D	D	D	D	D	D	D	D
	11	**35**, **45**, 46	49	54	64	82	85	99	114	107	122	D	D	D	D	D	D
	12	31	34	40	52	66	79	87	99	98	112	110	142	×	×	×	×
	13	30	31	38	46	67	74	83	94	96	116	110	137	×	×	×	×
	14	**27**,38	30	37	45	62	68	79	97	104	115	112	142	×	×	×	×
	15	**40**,47	38	47	53	63	70	83	100	92	115	114	142	×	×	×	×
	16	39	50	57	65	76	80	95	117	107	134	132	159	×	×	×	×
Received medulla	17	43	43	51	69	74	82	89	100	90	105	102	142	×	×	×	×
	18	35	33	39	57	66	73	83	97	92	102	104	140	×	×	×	×
	19	37	38	43	62	71	**80**	D	D	D	D	D	D	D	D	D	D
	20	36	35	42	60	63	74	83	96	93	107	97	107	×	×	×	×
	21	**38**,41	35	43	63	64	80	90	103	107	107	D	D	D	D	D	D
	22	**37**,44	50	56	72	70	**92**	D	D	D	D	D	D	D	D	D	D
	23	**30**,28	30	38	53	61	**65**	D	D	D	D	D	D	D	D	D	D
	24	**33**, **35**,41	36	37	44	51	67	69	78	80	D	D	D	D	D	D	D
	25	**35**,41	39	48	64	65	**77**	D	D	D	D	D	D	D	D	D	D
	26	42	35	42	60	70	76	80	85	99	D	D	D	D	D	D	D
	27	**36**,38	30	38	55	57	66	70	75	81	D	D	D	D	D	D	D
	28	39	36	44	63	70	78	D	D	D	D	D	D	D	D	D	D
	29	41	Rat 29 transferred to cage 24 when rat 24 died														
	30	46	Rat 30 transferred to cage 11 when rat 11 died														
	31	28	Rat 31 transferred to cage 23 when rat 23 died														
	32	41	Rat 32 transferred to cage 10 when rat 10 died														

Bold faced figures indicate that animal died.
D indicates that animal died or was killed.
× indicates that animal was living but was not weighed on that date.

These experiments indicate that whatever the substance or substances in the fresh gland which caused death, it probably was not adrenalin at least in its pure form. There were the possibilities, however, that death might have resulted from the decomposition products of adrenalin; that adrenalin was absorbed as it existed in the fresh gland while it was not absorbed as it existed in the extract; or that death might be due to other decomposition products of the fresh gland or other unknown substances present therein.

Feeding of decomposed (oxidized) adrenalin solution. A solution of a 1:1000 (Parke-Davis & Co.) adrenalin chloride was placed in an open beaker and exposed at room temperature for three days. The only macroscopic change in the appearance of the solution was a change in color from a nearly colorless solution to a deep pink. On 4/14/24 rats 21 and 26 weighed 107 and 99 grams respectively. Rat 26 was given 1 cc. of the oxidized adrenalin solution subcutaneously which resulted in convulsions in thirteen minutes and the animal died two minutes later. Rat 21 was given 2½ cc. of this same solution by stomach tube and no demonstrable effect was noted. This experiment was repeated several times with identical results.

To test the possibility of death in the series receiving whole gland, being due to decomposition products in the gland itself, a rat weighing 107 grams was starved twenty-four hours and was then given the adrenal glands from a rabbit removed immediately after the animal was killed. The two glands weighed one gram and were devoured within five minutes. There were no demonstrable effects even after several days. This experiment was repeated with fresh rabbit adrenals weighing 2.4 grams. A rat weighing 102 grams after thirty-six hours' starvation was given the adrenal glands from six small rabbits, the twelve adrenals weighing 4 grams. All were consumed within a few minutes. Three hours later the animal appeared somewhat depressed, but recovered within a short time and was normal the next morning. A rat weighing 105 grams was given the adrenal glands from twelve cats immediately after removal. The twenty-four glands weighed 4.7 grams, of which the rat consumed 3.25 grams. The animal exhibited no effects.

Feeding of desiccated adrenal gland (Lilly and Company.) It was found that when rats were hungry they would eat one or two 2-grain tablets of desiccated suprarenal gland without effect. To get them to eat more than this quantity it was necessary to mix it with their regular food. On 4/21/24 eight 2-gram tablets or 16 grains in all were ground in a mortar. The powder was mixed with a small quantity of food to which milk was added to bring it to a pasty consistency. Two rats weighing 107 grams each, after twenty-four hours' starvation, were each given a similar amount. Each rat ate about two-thirds of the mixture and the following day was

Bold faced figures indicate that animal died.
D indicates that animal died or was killed.
X indicates that animal was living but was not weighed on that date.

TABLE 2

	RAT	2/26	3/1	3/4	3/8	3/15	3/22	3/29	4/5	4/12	4/19	4/26	5/3	5/10	5/13	5/17
Controls	33	38	47	57	61	70	94	109	122	117	128	144	151	148	152	160
	34	39	49	61	63	79	97	115	125	128	137	150	163	167	168	169
	35	36	43	55	58	67	83	97	102	102	107	124	134	144	144	143
	36	42	51	63	66	79	97	116	118	121	129	146	164	171	171	172
	37	39	45	60	58	81	95	120	123	137	148	163	179	187	190	195
	38	37	45	60	62	74	89	106	118	114	122	136	150	156	160	163
	39	34	44	57	59	68	91	111	120	119	123	154	161	168	170	173
	40	44	53	66	72	81	102	114	126	127	141	153	168	172	171	170
	41	43	46	61	62	82	96	117	117	121	130	143	160	168	170	172
	42	41	40	52	54	73	93	101	104	109	122	139	155	163	165	167
Received cortex	43	35	41	52	64	82	91	112	130	126	141	158	177	192	198	211
	44	40	41	52	55	72	88	114	130	124	130	151	173	179	182	196
	45	34	41	53	57	75	93	113	126	124	136	156	177	195	201	210
	46	44	49	57	67	82	100	118	135	127	133	161	181	200	205	209
	47	38	41	51	57	68	93	94	107	109	112	132	160	167	170	175
	48	50	56	67	70	91	109	131	140	137	150	167	186	202	210	214
	49	41	49	60	68	84	105	127	141	140	143	165	185	195	200	205
	50	37	D	D	D	D	D	D	D	D	D	D	D	D	D	D
	51	38	43	53	61	79	98	110	121	121	128	150	167	178	180	182
	52	48	46	59	65	81	98	108	115	120	126	152	174	190	195	201
	53	43	46	60	60	74	97	116	128	124	148	162	146	168	175	182
	54	37	41	58	64	73	86	100	117	121	134	145	168	182	190	198
	55	44	42	51	55	73	91	117	131	133	142	165	185	200	200	200
	56	40	Accident			D	D	D	D	D	D	D	D	D	D	D
	57	35	38	48	54	70	92	110	124	122	126	147	162	179	182	186

| | | |

very sick. One rat recovered, but the other died in convulsions. The experiment was repeated with rats weighing 122 and 112 grams respectively. Each rat was given five 2-grain tablets or a total of 10 grains. Neither of them ate the entire amount, but the rat which ate the greater quantity became very sick and died in about eighteen hours. The other rat was but slightly affected. At the same time we succeeded in getting a rat weighing 134 grams to eat 2½ tablets of the desiccated preparation or a total of 5 grains. There were no symptoms. The fatal dose of this preparation of desiccated suprarenal gland for rats weighing around 100 grams is probably slightly under 10 grains, and the dose which produces symptoms more than 5 grams.

There is, then, something in the desiccated preparation of adrenal glands as well as in the fresh whole gland which will cause gastro-intestinal symptoms and death in the white rat. We feel sure that this substance is not adrenalin.

Feeding of autolyzed beef adrenal gland. While the fresh glands received from Swift and Company gave no indications of having undergone autolytic changes, it was still possible that changes might have occurred which could not be detected. This was suggested by the fact that rabbit and cat adrenal glands fed to rats immediately after their removal, produced no symptoms and in only one case a slight depression.

To determine the effect of autolytic products of beef adrenal glands, a portion of the ground whole gland was placed in an incubator at 37.5°C. for 24 hours. At the end of this period the material had changed to a brownish green color and had a very obnoxious odor, showing that there was putrefaction as well as normal autolysis. Extracts were made from this material with physiological salt solution. The bacteria were removed by passing the fluid through a Berkefeld candle no. 4. Similar extracts were made from autolyzed adrenal cortex and from autolyzed dog's brain tissue. These extracts were then tested on guinea pigs weighing 250 grams by intraperitoneal injections. Doses of ½ to 1 cc. were used. In all cases the extracts were made with 1 cc. of physiological salt solution per gram of tissue. Following the injections, fluid gradually accumulated in the abdomen of the animals and death followed in from three to eight hours, when autolyzed gland extracts were injected, while no symptoms were noted with the brain extract (table 3). A pup weighing 1770 grams was given 25 cc. of an extract of autolyzed whole adrenal gland by stomach tube. The animal became nauseated and vomited in about 45 minutes, after which he appeared normal.

Rats were then given some of the autolyzed whole gland extract by stomach tube. Two rats were each given 2½ cc. with no symptoms in one, but the other appeared somewhat depressed for a short time. Another rat was given 1 cc. of this extract intraperitoneally which was followed by

TABLE 3

ANIMAL	WEIGHT	MATERIAL INJECTED	TIME	REMARKS
Guinea pig 1	250	1 cc. Berkefeld filtrate of autolyzed adrenal medulla given intraperitoneally	2:32 p.m.	Died 10:55 p.m. (8 hours 23 minutes)
Guinea pig 2	260	0.5 cc. Berkefeld filtrate of autolyzed adrenal medulla given intraperitoneally	2:35 p.m.	Died 1 p.m. next day (22 hours 28 minutes)
Guinea pig 3	250	1 cc. Berkefeld filtrate of autolyzed whole adrenal given intraperitoneally	3:15 p.m.	Died 7:15 p.m. (4 hours)
Guinea pig 4	260	5 cc. Berkefeld filtrate of autolyzed whole adrenal given intraperitoneally	3:16 p.m.	Died 6:16 p.m. (3 hours)
Guinea pig 5	255	1 cc. Berkefeld filtrate of autolyzed cortex of adrenal given intraperitoneally	3:15 p.m.	Died 7:30 p.m. (3 hours 15 minutes)
Guinea pig 6	250	1 cc. Berkefeld filtrate of autolyzed dog's brain given intraperitoneally	3:30 p.m.	No effect
Guinea pig 7	250	1 cc. saline extract of fresh whole adrenal given intraperitoneally	4:19 p.m.	Died 6:45 p.m. (2 hours 26 minutes)
Guinea pig 8	250	1 cc. saline extract of autolyzed adrenal cortex given intraperitoneally	4:16 p.m.	Died 7:30 p.m. (3 hours 14 minutes)
Guinea pig 9	250	1 cc. saline extract of autolyzed adrenal medulla given intraperitoneally	4:15 p.m.	Died 4:31 p.m. (16 minutes)
Guinea pig 10	250	1 cc. saline extract of autolyzed whole adrenal given intraperitoneally	4:14 p.m.	Died 4:20 p.m. (6 minutes)
Guinea pig 11	250	Same as guinea pig 10 only injection was given subcutaneously	4:25 p.m.	Died 11 p.m. (6 hours 35 minutes)
Dog 1*	1,770	25 cc. saline extract of autolyzed whole adrenal given by mouth	10:05 a.m.	Vomited 10:50 a.m.

* This animal showed no other symptoms after vomiting. At 8:05 p.m. the same day this animal was given seven fresh beef adrenal glands to eat and at 9 p.m. vomited again but remained normal thereafter.

convulsions and death in thirty-two minutes. To determine if the adrenalin content of the extract could account for the death in this animal, four rats were given 0.25 cc., 0.5 cc., 1 cc. and 1.5 cc. respectively of a 1:1000 solution of adrenalin chloride intraperitoneally. The three rats receiving the largest doses died in from seven to ten minutes. The rat receiving 0.25 cc. died in seven hours. Four guinea pigs weighing 250 grams were given similar doses and only the animal receiving 1.5 cc. died after eight hours had elapsed. The other three animals were normal after forty-eight hours.

The adrenalin content of the extract of autolyzed adrenal gland could easily account for the death of the one rat in so far as we are able to judge from these experiments.

The lipoid content of the adrenal gland was next considered. The lipoids were extracted with equal parts of ether and chloroform, filtered and then evaporated to dryness. The resulting yellow residue was taken up in equal parts of liquid petrolatum. The injection of 1 cc. of this product subcutaneously into guinea pigs weighing 250 grams caused death in about twenty-four hours. Physiological tests on dogs for the presence of pressor substances in these extracts showed that they contained them in very small amounts. This does not exclude the presence of adrenalin. However, if the toxic substance contained in the desiccated gland was lipoidal in nature, it would have been largely removed in their preparation. That these desiccated products are not entirely degreased can be easily shown by dissolving the tablets in water and then extracting with ether. A very small amount of lipoid material remains in the ethereal fraction. We do not believe it is sufficient to account for the toxic reactions exhibited by rats which have been fed desiccated gland.

The question of bacterial decomposition of the gland was next considered. Two-hundred grams of fresh whole gland were repeatedly extracted with twice this volume of water and each extract was tested for pressor substances on dogs. This method required twenty-three extractions to entirely exhaust the gland tissue of its blood-pressure-raising substances. Only 60 grams of tissue remained and this material was placed in a sterile flask with a broth culture of the normal bacterial flora prepared from the rats' upper intestine (anaerobes not included).

After incubation for 24 hours at 37.5°C. the material was filtered through paper and then passed through a Berkefeld. This filtrate given by stomach tube to rats produced severe symptoms, but not death. The same filtrate given to rats by the intraperitoneal route caused convulsions and death in about thirty minutes.

Feeding of fresh beef adrenal cortex. Rats 43 to 57, after several days of observation on the standard diet, were fed with one gram of fresh beef adrenal cortex each day in addition to the regular diet. The death of

one animal on the second day requires no consideration as no symptoms were noticed in any of the other animals in this series. They ate the gland readily and seemed to thrive on it. Beginning with the second week the amount of cortex was increased to three grams which amount was given daily for the following ten weeks. Rarely was a trace of gland ever noticed in the cage at the next feeding time. The gain in weight paralleled the control animals for the first seven weeks. Beginning with the eighth week the average weight of the cortex series exceeded that of the control series by one tenth of the body weight. This was maintained and slightly increased to the end of the experiment (table 4).

At the end of the experiment the animals of the control series and the cortex series were killed by a blow on the head, and the testes, spleen and

TABLE 4

AVERAGE WEIGHT OF CONTROL RATS	AVERAGE WEIGHT OF CORTEX-FED RATS	AVERAGE GAIN IN WEIGHT OF CONTROL RATS	AVERAGE GAIN IN WEIGHT OF CORTEX-FED RATS	DATE
grams	*grams*	*grams*	*grams*	
$38\frac{5}{8}$	$40\frac{1}{4}$			2/26
$62\frac{3}{8}$	$61\frac{3}{4}$	24	21	3/4
$74\frac{7}{8}$	$76\frac{5}{8}$	12	15	3/15
$93\frac{1}{2}$	$95\frac{3}{5}$	19	19	3/22
111	$113\frac{1}{5}$	18	18	3/29
$120\frac{1}{2}$	$126\frac{7}{5}$	9	9	4/5
$120\frac{3}{8}$	$124\frac{1}{2}$	0	−2	4/12
$129\frac{3}{8}$	$134\frac{7}{5}$	9	10	4/19
$146\frac{1}{2}$	$154\frac{9}{5}$	17	20	4/26
$158\frac{1}{4}$	$172\frac{5}{5}$	12	18	5/3
$166\frac{1}{5}$	$186\frac{9}{5}$	8	14	5/10
$179\frac{1}{5}\frac{7}{5}$	$197\frac{9}{5}$	13	11	5/17

adrenal glands were carefully removed. They were placed immediately into weighed amounts of fixing fluid to prevent any possibility of loss of weight by drying. One of us removed all of the organs to insure a uniform technique, while the other determined the weights of each organ (table 5).

Discussion. From the data presented dealing with the feeding of fresh whole adrenal gland, it is observed that 45 per cent of the animals died when fed as much as one gram of the gland. A number of the other rats were depressed, but none of the animals ate all of the gland given to them. Probably death did not occur in these animals due to the small amount of the gland which was consumed. After eating small amounts of the gland most of the animals refused to touch it at subsequent feedings. Administration of the gland was successful when it was mixed with the diet.

When considerably larger amounts of rabbit and cat adrenals were fed

to rats immediately after removal, no severe symptoms resulted. This would seem to indicate that some autolysis had taken place in the glands received from the packing house, in spite of their having been kept at a very low temperature. Extracts of autolyzed beef adrenal glands were prepared and when administered to guinea pigs intraperitoneally, produced

TABLE 5

	RAT	WEIGHT OF RAT ON MAY 17	WEIGHT OF ADRENALS (2)	WEIGHT OF SPLEEN	WEIGHT OF TESTES (2)
		grams	*mgm.*	*mgm.*	*grams*
Controls	33	160	23	420	1.678
	34	169	27	352	1.639
	35	143	23	355	1.672
	36	172	20	473	2.046
	37	195	25	389	2.174
	38	163	24	429	1.900
	39	173	20	423	1.661
	40	170	25	390	1.638
	41	172	17	477	2.010
	1	177	21	431	1.738
	2	188	22	442	1.926
	42	167	22	348	1.844
	3	181	24	385	1.998
	4	189	28	390	1.470
	5	178	16	398	2.104
	6	183	23	311	2.123
	7	179	21	347	2.046
	8	180	18	375	1.938
Received adrenal cortex	43	211	25	525	2.007
	44	196	23	511	2.035
	45	210	23	475	2.125
	46	209	21	430	1.953
	47	175	16	405	1.856
	57	186	20	368	1.954
	48	214	24	565	2.129
	49	205	23	456	2.650
	51	182	24	722	1.920
	52	201	24	480	1.958
	53	182	23	569	2.111
	54	198	28	416	1.905
	55	200	25	432	2.042

death in from three to eight hours. The same extract when given to young pups by stomach tube produced nausea and vomiting in about 45 minutes. When this extract was administered to rats by stomach tube only mild symptoms were produced. When injected into the peritoneal cavity convulsions and death followed in about thirty minutes.

Control rats given 0.5 cc. to 1.5 cc. of adrenalin chloride (1:1000 solution) intraperitoneally died within ten minutes. A rat receiving 0.25 cc. of this solution lived for seven hours. Thus if the extract of the autolyzed tissue contained adrenalin, and it is assumed that the adrenalin content caused death in these animals, it was present in a quantity somewhat larger than 0.25 cc. of a 1:1000 solution. Control guinea pigs were given adrenalin chloride in the same doses and only the animal receiving the largest dose died within eight hours. The other pigs were alive and apparently normal even after forty-eight hours.

These experiments indicate that adrenalin was not solely responsible for the deaths in the animals receiving extracts of autolyzed adrenalin gland, since it required much larger doses of adrenalin chloride than could possibly have been present in the autolyzed tissue extracts, to produce effects in control animals.

By the colorimetric method of Folin, Cannon and Denis (4) the amount of adrenalin in each cubic centimeter of the autolyzed tissue extract was found to be 0.17 mgm. The control animal which lived for seven hours received 0.25 cc. of a 1:1000 solution of adrenalin chloride or 0.25 mgm. This animal did not have the accumulation of fluid in the abdomen characteristic of the animals given the autolyzed adrenal extract. While we do not attach a great deal of significance to this slight difference in amount of adrenalin as determined by the colorimetric method, still the difference in the symptoms produced suggests some factor other than adrenalin to be the cause of death.

The experiments with desiccated adrenal gland are in accordance with this view. That is, when rats fed doses of desiccated adrenal gland varying from five to ten grains, like symptoms or death resulted. We are not surprised at these results since desiccated glands are prepared from fresh whole glands oftentimes with less care being taken for their preservation than was taken with the fresh glands used in these experiments. It is conceivable that some of the autolytic products are present in the desiccated preparation. Indeed, Rowntree (5) recently reports gastro-intestinal symptoms in patients with Addison's disease, when treated with desiccated adrenal gland. The symptoms in our animals resemble those described by this author.

Extracts of whole adrenal gland, after exhaustion of pressor substances which had been incubated for 24 hours at 37.5°C. with the normal intestinal bacterial flora of the rat, produced severe symptoms but not death when administered to rats by stomach tube. However, this extract produced death in a rat in thirty minutes when given intraperitoneally. It is apparent then that there is some other substance or substances than adrenalin present in these extracts which will cause symptoms or death depending upon the route of administration and the amounts given.

From the feeding experiments with fresh adrenal cortex we were impressed with the eagerness with which the animals ate the cortical tissue as compared with the fresh whole gland or medullary portions. The animals gained in weight from the beginning while the animals receiving the fresh whole gland or medulla gained very little or lost in weight. At the end of the experiment the average weight of the control animals was 180 grams as compared with 198 grams for the cortex fed animals. That is, the rats receiving adrenal cortex were 9.1 per cent heavier than the control animals. The average weight of the spleens of the control animals was 396 mgm. as compared with 488 mgm., the average weight of the spleens of the cortex fed animals. The spleens in the experimental series were 18.9 per cent heavier than the spleens of the control animals. This is not in accord with the findings of Hoskins when dried suprarenal gland was fed. However, we do not attach much significance to the variation in the weight of this organ. The average weight of the testes of the control series was 1.858 grams as compared with an average weight of 2.416 grams for the cortex fed series. Here we note an increase of 21.5 per cent in the weight of the testes of the cortex fed animals. Microscopic studies did not reveal a detectable difference between the testes of the animals of each series.

The average weight of the adrenal glands of the control animals was 22.16 mgm. as compared with an average weight of 23 mgm. for the cortex fed animals. The variation in the weights of the adrenal glands was from 16 mgm. to 28 mgm. Though these weights were obtained with the utmost care, we attach no significance to these variations since the glands are so small and the variations so slight. In fact we do not wish to emphasize the difference in weights we have found for the different animals of the control and cortex fed series, but we have found it interesting to compare them with those reported by Donaldson (6) and find that the weights given for our normal animals are very close to those given by him. The increased weight of the testes in the cortex fed animals seems to justify the long thought of relation between these two organs.

CONCLUSIONS

1. Fresh whole beef adrenal glands, kept at a temperature below freezing, proved toxic to white rats when given in $\frac{1}{2}$ gram amounts.

2. Much larger doses of fresh whole adrenal glands removed from rabbits and cats and fed immediately to rats produced no serious effects.

3. Adrenalin chloride when given to the white rat by mouth in amounts as great as 10 cc. of a 1:1000 solution, produces no apparent effects.

4. When whole beef adrenal glands are exhausted of their adrenalin content and incubated with a culture of the intestinal flora of the white rat, and fed to rats, depression is noted.

5. Desiccated adrenal gland, when fed in amounts varying from 5 to 10 grains, will invariably produce untoward results.

6. Gastro-intestinal symptoms as described in man, following the administration of desiccated gland, are similar to those noticed in these experiments.

7. The cortex from beef adrenal glands may be kept as long as four days at a low temperature and fed to rats in amounts as large as 3 grams per day without ill effects. Only glands freshly extirpated should be used in feeding experiments.

8. Rats fed on cortex at the end of eleven weeks were 10 per cent heavier than the control animals.

9. The spleens of the cortex animals were 18 per cent heavier than the spleens of the control animals, while the testes were 21.5 per cent heavier than the testes of the control animals.

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STUDIES OF THE BRAIN STEM

X. The Conditions under which Extract of the Posterior Lobe of the Hypophysis Causes an Increase in Body Temperature

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Received for publication December 22, 1925

In the course of a series of experimental studies of the physiology of the basal nuclei of the brain, the writer was faced with the question as to whether or not any of the observed effects could be attributed to injury of the hypophysis. It is obvious that any surgical procedures in the basal regions of the third ventricle might involve some traumatism or circulatory disturbance of the hypophysis or its infundibular connections. If the hypophysis exerts any physiological action either through the blood stream or the nervous system, an extensive destructive lesion of the optic thalamus might therefore yield effects dependent on both nervous and endocrine factors. Particular attention has been devoted by many recent workers to the alterations in body temperature, polyuria and glycosuria that frequently follow lesions localized in this region of the brain. Decerebration with destruction of the thalamus destroys the ability of warm blooded animals to regulate body temperature (1), (2), (3), (4). Such animals at ordinary atmospheric temperatures usually exhibit sub-normal temperatures.

Several years ago the writer's attention was attracted to the clinical observations of Cushing (5) that sub-normal temperature was frequently observed in cases diagnosed as "hypo-pituitarism" and that the injection of extract of the anterior lobe in these cases was followed by a "thermic reaction" or elevation of body temperature. In the experimental work of the present writer on birds, surgical lesions had been made in the basal parts of the thalamus in the neighborhood of the infundibulum, sub-normal temperatures had followed, and because of the proximity of the lesion to the posterior lobe of the hypophysis, we subsequently injected extract of the posterior lobe into the experimental animals. Somewhat to our surprise, we found that this was followed by an elevation of the body temperature.

In pigeons rendered poikilothermic by destruction of the thalamus this is a very constant finding (6). The methods employed in this work are

as follows. Under ether anesthesia the cerebral hemispheres are exposed and then removed, taking care to do the minimum damage to the longitudinal sinus and the underlying basal meninges and optic thalamus. When hemorrhage is controlled, a clear view of the thalamus is obtained and this is then destroyed by the electro-cautery, taking care not to traumatise the hypophysis or optic lobes. With proper care this can be done, as shown by the subsequent absence of any muscular incoördination or forced movements and by autopsy examinations. Because of the high resistance

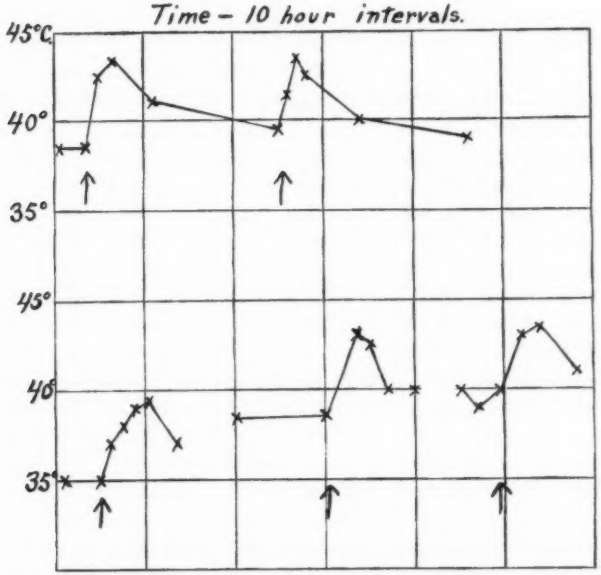

Fig. 1. Temperature reactions of four decerebrate poikilothermic pigeons following intraperitoneal injection of extract of the posterior lobe of the hypophysis. The normal body temperature of normal pigeons is about 41°C. Body temperature in degrees Centigrade. Abscissae mark 10-hour intervals. The arrows indicate the time of injections of the pituitary extract.

of pigeons to the ordinary contaminating microörganisms, infection is excluded as a cause of any subsequent temperature reactions. If the removal of the hemispheres and the thalamus be properly carried out, such a bird may be kept alive for several days or weeks, so as to recover from any possible temporary shock effects, provided the bird is kept at a constant atmospheric temperature of about 30° to 32°C. Such a bird is poikilothermic and its body temperature may usually be set at various levels by altering the atmospheric temperature. The body temperature

can be maintained at a nearly constant level if the atmospheric temperature be kept constant. If now an intraperitoneal injection of 0.2 to 0.5 cc. of an active extract of the posterior lobe of the hypophysis be given to such a bird there follows a rise in body temperature. In figure 1 are plotted a series of these thermic reactions as observed in a number of different birds. Bearing in mind that the normal body temperature of the pigeon is about 41°C. it will be observed that these thermic reactions can be obtained, both in birds whose body temperature is set at a normal level and in birds with subnormal temperatures. In normal pigeons administration of extract of the posterior lobe does not increase the body temperature above the limits of the possible diurnal variations.

Following these initial observations it was found that such a thalamic lesion in birds is followed by polyuria (7), as has been shown true for mammals by Camus and Roussy (8) by Bailey and Bremer (9) and others. This polyuria of birds is independent of water intake and is exhibited during fasting. The loss of water may be so severe as to lead to marked dehydration and the death of the bird (7). Since pituitary extract influences the secretion of urine and hence the water content of the body, and since the thalamic lesion destroyed body temperature regulation and caused polyuria, it was suggested that possibly a disturbance of the water balance of the body might be a primary factor in the thermic reaction caused by pituitary extract administration. With these considerations in mind it then became of interest to study specifically the conditions under which the extract of the posterior lobe causes a rise in body temperature. Four sets of observations have been made of the effects on body temperature of normal pigeons, under the following conditions:

1. Normal adult pigeons with normal diet and water.
2. Normal adult pigeons given dry mixed feed but no water.
3. Normal adult pigeons given water but no feed.
4. Normal adult pigeons given no feed or water and exposed to a hot dry atmosphere.

In this report by the term "thermic reaction" is meant a rise in body temperature after the injection of extract of the posterior lobe. The pituitary extract used in this work was the "surgical" extract prepared by the Lilly Company. The dosage was from 0.2 to 0.4 cc. injected intraperitoneally. Temperature readings were taken by inserting the thermometer bulb in the cloaca or into the oviduct. For any given bird, the temperature readings were always the maximal ones obtainable. This is an important detail for very different readings may be obtained by varying the distance the thermometer is thrust into the cloacal or oviducal cavities. Following the injection, the thermic reaction, if it occurs, usually begins within an hour.

1. The effects of pituitary extract in normal pigeons on a normal diet with water. The effects of pituitary extract in a normal bird under normal

conditions are described in table 1. It will be observed from this table that in the normal pigeon, pituitary extract causes polypnea, drooping of the wings, polyuria, and muscular weakness or unsteadiness. The body temperature does not rise above the upper limits of the normal diurnal variations which ranges from 41° to 43°C. The polypnea and drooping of the wings are part of the thermolytic mechanism of birds and it seems therefore that the tendency of pituitary extract to raise the body temperature is counteracted by the action of these thermolytic mechanisms. An hour or two after the injection, the body temperature very commonly falls to a level somewhat below the initial reading.

2. The effects of pituitary extract in normal pigeons given dry feed but no water. It has been shown in previous work (4) that on prolonged fasting the pigeon tends to become somewhat poikilothermic. The purpose of

TABLE 1

TIME	BODY TEMPERATURE	REMARKS
	°C.	
11:05	42.5	
11:10		0.35 cc. pituitary extract injected
11:20	42.3	Urination
11:45	41.6	0.4 cc. pituitary extract injected
11:55	42.3	Marked polypnea. Urination
12:15	42.9	Polypnea. Dribbles urine. Muscular weakness
12:30	42.7	
1:00	42.2	
2:00	41.4	
3:00	41.5	

this experiment was to attempt to produce a water deficiency in the body with minimal disturbances due to fasting. Under the conditions of dry feeding, temperature disturbances are known to occur (10). If the thermic reaction of pituitary extract is dependent on a certain degree of dehydration it seemed, a priori, that it should occur if administered to a dry-fed animal. The detailed record of such an experiment is given in table 2.

This experiment has been repeated on five adult pigeons. In each case of feeding the birds on dry mixed grain and giving them no water, it was found that after eight to twelve days, the intra-peritoneal injection of pituitary extract caused a distinct rise in body temperature to a maximum level, in this series, of 2 to 3°C. above the initial temperature readings and distinctly above the upper limit of the normal variations exhibited by resting birds. The results of the tests in these five pigeons are given in table 3.

The temperature reactions after injection of pituitary extract, in these

birds submitted to prolonged dry feeding, are very similar to those of birds in which the cerebral hemispheres and thalamus have been destroyed. This suggests that probably the fundamental cause is similar in the two cases, namely, an adequate degree of dehydration.

TABLE 2

DATE	TIME	BODY TEMPERATURE	REMARKS
		°C.	
November 2			Begin dry feeding
November 9	8:40	40.0	
	8:50		0.3 cc. pituitary extract
	9:20	39.8	
	9:30		0.3 cc. pituitary extract
	9:55	41.2	
	10:50	41.6	
	11:30	41.3	
November 10			Dry feeding continued, no water
November 12	9:00	41.6	
	9:05		0.35 cc. pituitary extract
	9:15	41.5	Urination
	9:35	42.6	Urination, wings drooped
	10:00	43.2	0.3 cc. pituitary extract
	10:10	43.3	Urination, wings drooped
	10:20	44.0	Slight hyperpnea, no polypnea
	10:55	44.3	
	11:35	44.0	
	2:45	41.4	
	3:00		Bird given feed and water

TABLE 3

INITIAL TEMPERATURE	MAXIMAL TEMPERATURE AFTER PITUITARY EXTRACT
°C.	°C.
42.2	44.0
42.0	44.0
42.1	43.3
42.0	43.8
41.5	44.2

3. The effects of pituitary extract on pigeons given water but no solid food. Eight adult pigeons were given water, but no food, for a period of eight to ten days, a period corresponding to the same time interval of dry feeding used in series 2, in which it was found that pituitary extract would cause a thermic reaction. After this period of total abstinence from dry food but with plenty of water supplied, pituitary extract was injected intraperitoneally in these birds with observations of the body temperature at fifteen-minute intervals. The results of this test are shown in table 4.

It will be observed from this table that in this series of birds which were given water but no solid food, pituitary extract did not cause a thermic reaction. There was a slight and distinct tendency for the body temperature to rise but this did not exceed the limits of diurnal variations. In these birds as in birds on a normal diet, a thermogenic action of pituitary extract is suggested by the appearance of dropping wings and flattening of the feathers against the body, reactions which are parts of the thermolytic system of birds. It seems that in the presence of ample tissue water the thermogenic tendency of the extract can be counteracted by the usual thermolytic means.

4. The effects of pituitary extract after deprivation of food and water and exposure to a hot dry atmosphere. If the thermic reaction is dependent on

TABLE 4

The effects on body temperature of injection of pituitary extract in eight pigeons given water but no food, for a period of eight to ten days

A slight thermogenic tendency is evident but the body temperature did not rise above the limits of normal variations.

BODY TEMPERATURE BEFORE INJECTION OF PITUITARY EXTRACT	AMOUNT OF EXTRACT INJECTED	MAXIMUM BODY TEMPERATURE AFTER INJECTION	REMARKS
°C.	cc.	°C.	
40.5	0.4	39.0	Death in 10 minutes
40.5	0.3	41.0	
40.9	0.2	42.0	
41.2	0.2	42.3	
41.2	0.2	41.8	Death in 1 hour
41.1	0.3	41.2	Death in 15 minutes
41.8	0.2	42.0	Death in 20 minutes
40.6	0.4	41.7	Death in 35 minutes

a certain degree of dehydration it would seem that if normal birds were deprived of water and kept in a warm dry incubator, then the subsequent administration of pituitary extract should be followed by a rise in body temperature. It is a well-known fact that under these conditions of water deprivation and exposure to dry heat, an exhaustion of the water reserves may lead to a rise in body temperature. In order to demonstrate a thermic effect due to the administration of pituitary extract, the period of exposure to dry warmth must not be too great else a rise in temperature may be attributed to heat exposure only; and if the period of exposure is too short a sufficient loss of water may not have occurred. Furthermore the water reserve may be different in different birds. Hence under these conditions the test of a possible thermic reaction due to the pituitary extract requires continuous watching of the test animals. Several tests of this character

proved inconclusive but in at least four instances positive results were obtained. The detailed records of two of these successful experiments are given in table 5.

From these experiments the conclusion seems justifiable that if a certain degree of dehydration is obtained by water deprivation and exposure to dry heat, the administration of pituitary extract will then cause a rise in body temperature.

Two other additional observations made in the course of this work are worthy of mention. It has frequently been observed that either after decerebation or after prolonged fasting, the birds seem much more susceptible to the toxic effects of pituitary extract than do the normal birds. This is shown very clearly in table 4. In many instances the birds have died in the observer's hand while the temperature reading was being made. It has been frequently observed that with the bird held in the hand so that the heart beat could be felt, the mechanical irritation of the cloacal, rectal or oviducal structures by the thermometer bulb was followed by sudden inhibition of the heart beat and death of the bird. This has been so frequent an occurrence after administration of the pituitary extract that it suggests that under the influence of this extract, the reflex of cardiac inhibition by rectal irritation is very much augmented.

TABLE 5

NUMBER OF PIGEON	DATE	TIME	BODY TEMPERATURE	REMARKS
			°C.	
56	June 22			Into a dry incubator, temperature 34°C. No food or water
	June 23			Pituitary extract injection causes no rise in body temperature
	June 24			Pituitary extract injection causes no rise in body temperature
	June 25	8:30	43.0	Pituitary extract injected
		9:00	44.8	
85	January 4			Fasting begins. No water
	January 7			Into incubator at 40° C. for five hours
	January 8			Into incubator at 40°C. for five hours. Body temperature rises to 44.0°C. Taken out of incubator. Body temperature falls
	January 8	9:00 p.m.	39.2	Inject pituitary extract
		11:35	44.1	Bird died while this temperature reading was being made

DISCUSSION. It is not certain how far this described relation between water loss and pituitary extract hyperthermia can be applied to mammals.

As yet we have made only a few preliminary experiments on rabbits, cats and guinea pigs, but these indicate that other factors must be considered. Simple water starvation of cats and rabbits followed by administration of pituitary extract, even in toxic doses, has not in these experiments been followed by a thermic reaction. What the missing condition may be, we cannot state. Three possible factors may be mentioned: for mammals a different method of producing an adequate dehydration may have to be employed; certain differences in nitrogen metabolism of mammals as compared with birds may have to be considered; and finally, possible differences in the effects of pituitary extract on the secretion of urine may play a part. Certainly in unanesthetised pigeons, pituitary extract causes diuresis, whereas in mammals it may exert an anti-diuretic effect. Further study of this problem is therefore required.

It is possible that the thermic reaction of birds induced by extract of the posterior lobe, though superficially resembling the thermic reaction described by Cushing as following administration of extract of the anterior lobe, may be of essentially different causation. In the light of the results herewith described it would be of interest to determine whether or not in clinical cases of involvement of the hypophysis, extracts of the posterior lobe may not sometimes yield thermic reactions. If this should be true, then, one would wonder whether or not the extract of the anterior lobe that yields a thermic reaction, may not be contaminated with the active principle of the posterior lobe.

However this may be, the experimental results cited above seem to show conclusively that in birds, if an adequate degree of water loss from the body is attained, the subsequent administration of extract of the posterior lobe will lead to an elevation of the body temperature.

SUMMARY

In normal adult pigeons on a normal diet, the intraperitoneal administration of extract of the posterior lobe of the hypophysis causes: first, a slight increase in body temperature, not exceeding the limits of the normal diurnal variations, and second, the appearance of polypnea and drooping wings, which are parts of the thermolytic mechanism in birds.

In pigeons which have lost excessive amounts of water from the body, extract of the posterior lobe frequently causes an increase of body temperature of 1 to 3 degeees Centigrade above the upper limits of the diurnal variations. Three methods of causing this loss of water or tissue dehydration have been employed; dry feeding, exposure of fasting birds to a warm dry atmosphere and the production of experimental polyuria in fasting birds by a destructive lesion of the thalamus. In all three conditions, the intraperitoneal injection of extract of the posterior lobe was followed by thermic reactions.

In unanesthetised pigeons under these conditions of dehydration and pituitary extract administration, fatal reflex cardiac inhibition frequently follows mechanical irritation of the cloaca, intestine or oviduct.

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THE EFFECT OF AMYTAL ANESTHESIA UPON THE RESPONSE TO INTRAVENOUS INJECTION OF GLUCOSE

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Received for publication December 23, 1925

In connection with our study of the response to intravenous injections of glucose in dogs, it was necessary to make tissue analyses at various periods of observation. This necessitated use of general anesthesia. Because of the hyperglycemia associated with the use of most general anesthetics, it was thought that amytal might be particularly valuable in this work.

Page (1923) has reported that amytal (iso-amyl ethyl barbituric acid) is capable of maintaining satisfactory surgical anesthesia without producing hyperglycemia. That there was not an associated generalized inhibition of normal carbohydrate metabolism was indicated by the fact that hyperglycemia was obtained under these conditions upon splanchnic stimulation or severe hemorrhage, and that insulin still produced hypoglycemia. Numerous observers have verified Page's findings as to absence of hyperglycemia, and have reported experiments dealing with carbohydrate metabolism on animals under the influence of this anesthetic. Cori (1925) has concluded that its use has no effect on carbohydrate metabolism, and has used it in the determination of tolerance for injected glucose in rats.

It is, however, not safe to assume from the observations recorded that the use of amytal is associated with no alteration of carbohydrate metabolism. It might be expected that more delicate methods of estimation of carbohydrate utilization would indicate an impairment of that function under the influence of any adequate anesthetic.

In a previous study by the authors (1925) it was observed that the response of any one animal to the continuous intravenous injection of glucose was quite constant, as judged by studies of blood sugar, hemoglobin, plasma pH and carbon dioxide content, glycosuria, respiratory quotient and heat production.

This report deals with a comparison of the response of dogs, with and without amytal anesthesia, to the continuous intravenous injection of glucose at the rate of four grams per kilogram of body weight per hour for periods of three to four hours. The methods of study used in this work have been described elsewhere (Boyd, Hines and Leese, 1925). Briefly

[1] Reported in abstract before the Society for Experimental Biology and Medicine.

they consist in the determination of the various factors before, during and after the injection period. Dogs were kept in cages on a standard diet. Blood sugar determinations were made by a micro Folin and Wu method upon blood from the marginal ear vein. Hemoglobin estimations were made according to Newcomer. Plasma CO_2 content was determined according to the method of Van Slyke and Cullen, and pH by the method of Myers, Schmitz and Booher. The blood for these determinations was drawn from the saphenous or jugular vein. Urine was collected by continuous catheterization and analyzed for sugar by the Folin and Berglund method. Air for determination of respiratory quotient and heat production was collected in a Tissot spirometer and analysed according to Haldane. A 30 per cent solution of glucose was injected in the saphenous

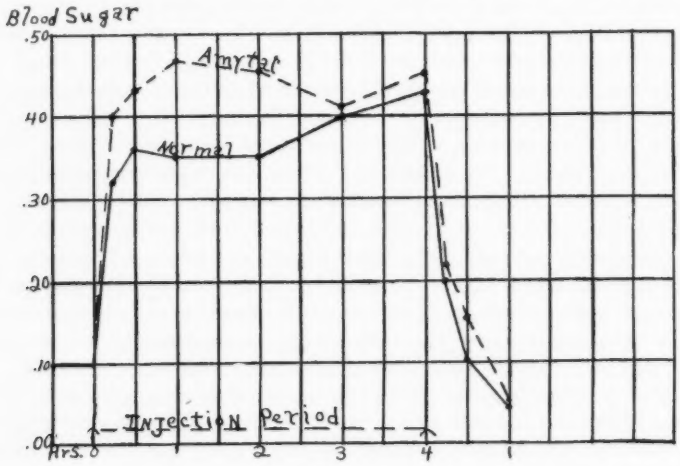

Fig. 1

vein by means of a Woodyatt pump. The amytal was administered by the subcutaneous or intraperitoneal route, as indicated in the protocols.[2]

Results. Amytal was without effect on the preliminary blood sugar values, but caused a greater degree of hyperglycemia during the period of glucose administration. This effect was most marked in the first and second hours of injection. The values tended to approximate each other more closely in the third and fourth hours. Figure 1 represents the average blood sugar values obtained from the group of animals studied, with and without amytal. The same animals were used in each case. The individual experiments are in harmony with the average curves. The return to normal blood sugar values in the post-injection periods was

[2] The amytal used was made available through the courtesy of the Eli Lilly Co.

delayed by the anesthesia in some experiments, while in others this effect was not noted.

Table 1 summarizes the degree of glycosuria in six different animals, with and without amytal. In cases where more than one experiment was performed on the same animal, the average was used in the table. In all cases anesthesia was associated with greater loss of sugar in the urine than obtained in the same animal under similar conditions without amytal. The extent of this increased glycosuria varies in different experiments, but for the group it averages 75 per cent higher than in the non-anesthetized animals.

TABLE 1

Percentage of injected glucose excreted in urine, based upon the percentage of total amount injected and eliminated up to the designated time

EXPERIMENT	END FIRST HOUR	END SECOND HOUR	END THIRD HOUR	END FOURTH HOUR	TOTAL
With amytal anesthesia					
609K&N	20.9	20.7	29.1	31.9	32.8
611E	12.1	10.4	16.2		17.2
612C	18.3	25.7	28.2		29.7
615E	23.0	28.1	31.7	31.2	32.2
616D	29.3	38.2	44.4	48.8	49.5
618C	21.8	30.7	34.3		34.6
Average....	20.9	25.6	30.6	37.3	32.7
Same animals without amytal anesthesia					
609	9.7	10.3	15.2	20.2	17.9
611	10.2	10.1	14.5		15.3
612	6.1	10.5	11.3		12.2
615	9.9	12.3	15.9		17.9
616	14.8	19.1	21.2	23.4	23.6
618	17.0	18.1	23.9		24.6
Average....	11.3	13.4	17.0	21.8	18.6

Table 2 summarizes the values for the respiratory quotient and heat production obtained from the several animals. The preliminary values tend to be slightly lowered when anesthesia is employed. During the injection the average increase in heat production amounts to 57 per cent as compared with 52 per cent for the same animals without amytal. These findings are quite consistent except in one experiment (612C) in which the respiratory quotient, heat production and body temperature fell during the course of the injection due to the extreme depth of the narcosis. This experiment is omitted from the averages.

No evidence was found that the anesthetic had lowered appreciably the

pH or CO_2 content of the plasma during the first two or three hours after its administration. However, values obtained at the end of the injection

TABLE 2

Respiratory quotients and calories per square meter body surface per hour. Based upon Rubner's constant in Meeh's Formula—11.2$\sqrt[3]{wt.\ in\ gm.^2}$

EXPERIMENT	PRELIM. R.Q.	PRELIM. HEAT	INJECTION PERIOD. R.Q.	INJECTION PERIOD. HEAT	POST INJECTION R.Q. heat 0–1 hour		POST INJECTION R.Q. heat 1–2 hours		POST INJECTION R.Q. heat 2–4 hours	
609K	0.79	36.6	1.01	62.2	0.98	43.7	1.07	41.9	0.90	41.7
609N	0.74	37.9	0.95	57.2			0.85	44.2		
611E	0.89	45.4	0.97	64.3	0.84	57.7			0.89	40.0
*612C	0.74	37.5	0.74	33.0					0.71	32.8
616D	0.71	40.1	0.97	66.6	0.86	42.6	0.84	37.8		
618C	0.83	41.8	1.08	64.7			0.96	42.9		
Average.....	0.79	40.4	1.00	63.4	0.89	48.0	0.93	41.5	0.90	40.8
Average of the same animals without amytal anesthesia.....	0.82	44.7	0.98	67.9	0.94	47.4	0.88	49.8		

* Omitted from average—note protocols.

TABLE 3

Blood plasma pH, CO_2 and hemoglobin relationships—determined before and at the end of the injection

EXPERIMENT	CO_2* Before	CO_2* End	CO_2* Difference	pH Before	pH End	pH Difference	Hb† End
609K	54	37	−17	7.36	7.32	−0.04	123
609N	55	38	−17	7.37	7.25	−0.12	110
612C	50	41	−9	7.36	7.19	−0.17	110
615E	40	36	−4	7.36	7.29	−0.07	104
616D	54	41	−13	7.38	7.28	−0.10	110
Average.....	50.6	38.6	−12	7.365	7.265	−0.10	111.4
Average based upon the same cases without amytal anesthesia.....	49.0	40.0	−9	7.357	7.317	−0.04	98.5

* CO_2 equal volumes per cent.

† Initial value of Hb equal to 100 per cent.

period showed an average fall of 0.1 pH as compared with 0.04 for the same animals when used as controls. (See table 3.)

DISCUSSION AND CONCLUSIONS. When amytal is administered to dogs in amounts sufficient to maintain light or deep surgical anesthesia, the

capacity to handle glucose is lessened. The blood sugar values are higher than in the controls. Unless the amytal dosage is large enough to cause a marked fall of body temperature and an upset of the chemical regulatory mechanism, the injection of glucose is associated with the usual rise of respiratory quotient and approximately the same increase of heat production. The reason for the diminished capacity to handle glucose is not evident. In order to determine the possible occurrence of changes in renal permeability, some estimations were made of the reducing substances present in the urine of dogs which had not been given glucose, but had received the standard diet. The anesthetized animals showed slightly greater excretion of reducing substances, but never great enough to reduce Benedict's qualitative reagent. Correlating this negative finding with the hyperglycemia associated with the injection, it would suggest that with amytal anesthesia the glycogenetic function is impaired.

These observations were made under different depths of anesthesia and at various intervals after administration of the anesthetic. Considerable variation exists in different experiments in the magnitude of the effects. All indicated a lessened ability to handle glucose. Therefore we feel that they are of sufficient degree to warrant the conclusion that an animal while under the influence of amytal does not exhibit its normal carbohydrate metabolism.

SUMMARY

Amytal anesthesia was found to lessen the ability of an animal to handle glucose when injected by the continuous intravenous method. This is evidenced by increased hyperglycemia and glycosuria, associated with slight lowering of the plasma pH.

PROTOCOLS. *Dog 609K*, young male bull terrier, weight 13.2 kgm. On standard diet. On March 19, 1925, was given 0.06 gram amytal per kilo intraperitoneally. Two hours later commenced four hour injection of 31.25 per cent glucose, 679 cc. in all. Animal under light surgical anesthesia during course of observation; slight shivering noted during early injection. During injection period passed 940 cc. urine, and rectal temperature rose 2.8°C.

Dog 609N. On May 19, 1925, same animal received 0.09 gram amytal per kilo subcutaneously. Degree of anesthesia was not sufficient, so four hours later an additional 0.02 gram per kilo was given intraperitoneally. Animal then developed moderately deep surgical anesthesia. Fifteen minutes later, started injection of 26.4 per cent glucose solution. After two hours, 859 cc. had been introduced; during that period 1013 cc. of urine were voided. Rectal temperature rose 1.9°C. during injection. No shivering was observed.

Dog 611E. Female airedale, weight 11.0 kgm. On standard diet. February 26, 1925, at 6:30 a.m., was given 0.06 gram amytal per kilo intraperitoneally. Two hours later animal was under light surgical anesthesia. During three hours' injection, received 420 cc. of 32 per cent glucose solution. Rectal temperature rose 2.9°C. One and a half hours after injection was terminated, the total urine output had been 340 cc.

D og 612C. Female Eskimo, weight 11.8 kgm. On standard diet. Two and a half hours after receiving 0.11 gram amytal per kilo subcutaneously, animal was in very deep surgical anesthesia. Rectal temperature fell during experiment to 35.2°. The pH fell from 7.36 to 7.18. Heat production and respiratory quotient were also lowered, in spite of the fact that glucose was being administered and retained in considerable amounts. Because of the depth of anesthesia, it is probable that the heat-regulating mechanism was affected. For this reason, the respiratory data were discarded. During the course of three hours and thirteen minutes' injection, animal received 513 cc. of 30.3 per cent glucose solution, and passed 746 cc. urine.

Dog 615E. Spitz female, weight 7.5 kgm. On standard diet. Three hours after receiving 0.06 gram amytal per kilo intraperitoneally, moderate anesthesia resulting, injection was begun. During four hours it received 398 cc. of 28.4 per cent glucose. Two hours after termination, total urine output had been 447 cc.

Dog 616D. Female terrier, weight 8.6 kgm. On standard diet. Given 0.06 gram amytal per kilo intraperitoneally. Eight hours later there was satisfactory anesthesia. Injection begun. In 4 hours received 411 cc. of 28.4 per cent glucose solution. Urine output was 650 cc. Rectal temperature rose 0.9°C.

Dog 618C. Female shepherd, weight 13.6 kgm. Received 0.11 gram amytal per kilo subcutaneously. During three hours' injection received 530 cc. of 31 per cent glucose, and passed 670 cc. urine. Rectal temperature rose 2.1°C.

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THE EFFECTS OF X-RAY ON THE PANCREAS

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Received for publication December 29, 1925

The results of exposure of different parts of the body to x-ray have been far from uniform. The difference in dosage will not explain the variations satisfactorily. Often a large surface of the body has been radiated and the cause of death ascribed to the injury of some gland contained in the exposed area. Thus Holfelder and Peiper (1) ascribe the cause of death to adrenal injury when the lumbar region is radiated with 60 per cent of a skin unit dose. The work of Martin et al. (2), seems to disprove the above results. That is, when much larger doses were applied directly to the adrenal tissue, very little change was noted.

Ivy et al. (3), report a hypersecretion of gastric juice followed by a hyposecretion when a human erythema dose was applied to the abdomens of dogs with Pawlow pouches. Death resulted in 6 to 14 days. In one dog there was a marked fibrosis of the pancras. Swann (4) reports a sensitization of the autonomic nerves with short exposures to x-rays and depression with prolonged radiation.

The only conclusions that are permissible seem to be that there is a big difference in sensitiveness of the different tissues to x-rays. In some cases there seems to be a stimulation to hyperactivity followed by a response below normal.

It was our purpose in these experiments to determine the effect of exposure of the *isolated* pancreas to measured doses of x-ray. Four cases are here reported as an introduction to this type of work. Numerous experiments of a similar nature are in progress.

METHOD. Dogs were used in all experiments. The animals were prepared as for the usual pancreatectomy operation. The head and body of the pancreas were completely removed. Beginning at the base of the duct of Sanatorini, the main pancreatic duct in the dog, the pancreas tissue was carefully removed for about 3 cm. from the base of the duct. The duct joining the main and accessory ducts was ligated. Thus was left sufficient of the tail of the pancreas to make up about one-third of the entire gland, with the main duct intact. The blood vessels going to the tail permitted the bringing of the remaining portion of the gland to the outside for radiation.

Lead plates, $\frac{1}{4}$ inch in thickness, were so shaped to conform to the dog's body beneath the pancreas and prevent any primary rays from reaching the animal. Sterile towels were placed about the lead plates to maintain asepsis and to absorb the secondary rays from the metal plates. The blood supply to the gland was not interfered with.

The following technic was used for treating the pancreas:

140 kilovolt chrest.
5 milliamperes.
4 mm aluminum filter.
8 inch focus skin distance.

The 10-inch transformer (Standard X-Ray Company) was used for the tests; all treatments were given with limited capacity.

Under these conditions, $4\frac{1}{2}$ minutes' exposure produced a human erythema dose, represented by 450 R units of primary rays with an effective wave length of 0.27 A U. Three, four and five erythema doses were applied to the surface of the tail third of the pancreas. The thickness of tissue was so little, and the radiation sufficiently hard as to consider the whole tissue treated with that dosage. The exposure time amounted to $13\frac{1}{2}$, 18 and $22\frac{1}{2}$ minutes.

Following the radiation an accurate record was kept of the food eaten by each animal. Water was given ad libitum after there was complete recovery from the operation. The twenty-four hour sample of urine was collected in a bottle rinsed with thymol. The sample was closed at 8:00 a.m. each day, and the volume recorded. Benedict's quantitative method was used for the sugar determinations.

PROTOCOL: *Dog 1.* Weight = 14 kgm.

DATE	REMARKS	URINE	PER CENT SUGAR	TOTAL SUGAR
		cc.		*grams*
March 31	a.m. Removed $\frac{3}{4}$ pancreas and rayed tail 13 minutes. Under ether 2 hours			
April 1	Appears fine. 4 p.m. drank 250 cc. milk, 10 p.m. drank 500 cc. milk	None		
April 2	Very active. Water ad libitum	130	0.26	
April 3	a.m. Appears fine. Urine lost			
April 4	Condition excellent	600	Negative	0
April 5	Condition excellent	725	Negative	0
April 6	Condition excellent	1,510	Negative	0
April 7	Wound healed	1,200	Negative	0
April 8		1,430	Negative	0
April 9	Polyphagia	1,070	Negative	0
April 10		825	Negative	0
April 11		550	Negative	0
April 12	Condition excellent	1,030	0.18	1.8
April 13	Condition excellent	1,010	0.19	1.9

PROTOCOL; *Dog 1.* Weight = 14 kgm.—*(continued)*

DATE	REMARKS	URINE	PER CENT SUGAR	TOTAL SUGAR
		cc.		*grams*
April 14	Condition excellent	1,425	0.5	7.1
April 15	Very active	900	1.3	11.7
April 16	Marked polyphagia	1,030	2.5	25.7
April 17		1,245	2.5	31.1
April 18		1,010	3.9	39.4
April 19	Condition fine	1,010	2.5	25.3
April 20		Not determined		
April 21		940	2.1	19.7
April 22		1,000	Not determined	
April 23	Not as active as usual	940		
April 24		1,400	0.83	13.8
April 25		680	0.9	6.1
April 26	Weight = 12.5 kgm.	860	0.61	5.2
April 27	Appears depressed	580	0.47	2.7
April 28		560	0.3	1.6
April 29	Ate rather eagerly	820	Negative	0
April 30	Walks about but did not eat	800	Negative	0
May 1	Dead	250	Negative	0

Autopsy: Liver—normal in appearance.

Thyroids—slightly enlarged.

Pancreas—weight of rayed portion of pancreas slightly more than 4 grams. Around the periphery of the gland were numerous small round nodules about 3 to 6 mm. in diameter. The pancreas was more solid than normal. The nodules appeared like regenerated tissue.

Stomach—at the pyloric end was a small ulcer.

Post-mortem examination did not furnish a satisfactory explanation for the cause of death.

Microscopic examination: Pancreas—the nodules at the periphery proved to be regenerated pancreas tissue—both islet and acinar tissue. The central portion of the rayed gland consisted entirely of a mass of fibrous tissue with a few interspersed areas of pancreas tissue which showed degenerative changes.

Dog 2. Male, weight = 11 kgm.

April 7, removed $\frac{2}{3}$ of pancreas and rayed the tail of the pancreas 18 minutes—4 erythema doses. The dog made an uneventful recovery. The amount of urine was measured daily and examined for sugar several times a week. Occasionally there would be a trace of sugar, but never more than a gram per day until July 26, 110 days following the exposure to x-rays. From the 110th to the 115th day a trace of sugar appeared in the urine, but the total never exceeded 5 grams on any one day. Following this period of temporary glycosuria there was not a trace of sugar in the urine. The dog did not have a polyuria or polyphagia at any time during the experiment. The weight and condition of the dog were the same on the 157th day after radiation as at the time of operation. It became necessary to terminate the experiment at this time.

Autopsy: The tail portion of the pancreas which has been rayed had disappeared entirely. At the base of the main duct was 275 mgm. of regenerated pancreas tissue, and at the base of the accessory duct was 100 mgm. of similar tissue. No gross pathology was noted in the other organs.

Microscopic examination: The regenerated pancreas tissue consisted apparently of the usual ratio of islet and acinar tissue. No pathological changes were detected in the other organs.

Dog 3. Female airedale, weight = 12 kgm.

June 18, removed head and body of pancreas. Rayed the tail 18 minutes—4 erythema doses. The dog made an excellent recovery. From the 7th to 9th days following the operation there was a trace of sugar present in the urine, but did not exceed 2.6 grams on any day. The dog was very active and ate about 2 pounds of meat daily. On the 5th day following the operation the output of urine was increased three times the normal amount for this animal and this new level maintained throughout the experiment. Sugar did not appear in the urine in appreciable amount until the 35th day following the operation, when 6.6 grams were excreted. The amount of sugar in the urine increased steadily until the amount of 43.5 grams was reached on the 42nd day following radiation. Then the output of sugar decreased gradually until the urine was free from sugar at death, the 60th day after exposure to x-ray. The dog appeared very active throughout and ate greedily up to the end. There was a gradual loss of weight from 12 kgm. to 7 kgm. during the 60 days of the experiment. The polyuria appeared on the 4th day following radiation, but sugar was not detected until the 35th day. The normal average output of urine for 5 days was 507 cc. Beginning with the 5th day after the operation the volume of urine voided averaged 1595 cc. per day for the remaining 56 days. The average per day during the latter part of the experiment, when sugar was present, was slightly lower than during the 35 days when no sugar was present in the urine.

Autopsy: Animal emaciated. The rayed portion of the pancreas had entirely disappeared. At the base of the main duct was 1 gram of regenerated pancreas tissue. The liver did not appear like a diabetic liver. No gross pathological changes were observed.

Microscopic examination: Pancreas—the regenerated tissue appeared like normal pancreas tissue. Liver—slight fatty infiltration. Other organs appeared normal.

Dog 4. Female collie, weight = 12.4 kgm.

April 28. Removed $\frac{3}{4}$ of the pancreas and rayed the tail $22\frac{1}{2}$ minutes—5 erythema doses. The dog made an uneventful recovery. On the day following the operation the urine contained a trace of sugar. The dog was kept on a carefully weighed diet of ground lean meat mixed with a box of Lister's sugar-free diabetic flour each day. Water was given ad libitum. The output of glucose in the urine gradually increased from the 2nd day following the operation until the 8th day. At this time the output of sugar exceeded the intake. That is, complete diabetes was reached on the 8th day following the operation. This condition was maintained to the end of the experiment. There was a rapid loss of weight as in a typical case of pancreatic diabetes in the dog. The symptoms of polydypsia and polyuria were not present at any time. The animal appeared very active and in apparent good health until the development of extreme emaciation. All food given the animal was devoured eagerly, even on the last day of life. The average daily output of urine was 280 cc. which agrees very closely with the normal daily output of 370 cc. before operation. The percentage of glucose in the urine was always high, the highest value being 8.8 per cent. Death occurred on the 68th day following radiation. The weight of the animal on the day preceding death was 7.2 kgm.

Autopsy: Animal emaciated. Pancreas—no regenerated or accessory pancreas tissue found. Kidney—apparently normal. Liver—typical diabetic liver. Spleen—very small—6 cm. long by 1 cm. wide. Thyroids—about 1 cm. in length and 0.3 cm. in diameter. Lungs—normal.

Microscopic examination: Liver—very marked fatty infiltration. Very few normal liver cells present. All other tissues showed changes resembling those usually found in a dog dying of diabetes.

Discussion. From the foregoing statements it is evident that the effects of radiation of the pancreas can be followed without the complications of other tissues being involved. In the case of dog 1, when three erythema doses were applied, there was no apparent change until the 13th day following radiation, at which time a trace of sugar appeared in the urine. The amount of sugar in the urine increased to 40 grams per day within a week. Then the glycosuria decreased gradually until at the end of ten days the urine was sugar free. The dog appeared in fine condition at this time. Death occurred three days later from an unknown cause. The recently developed nodular tissue about the border of the rayed pancreas appeared like regenerated pancreas. This was confirmed by microscopic examination. The nodules were normal pancreas tissue and presented a marked contrast to the rayed portion of the gland which consisted almost entirely of fibrous tissue with a few interspersed contracted lobules showing marked degeneration. The regenerated tissue at the border of the rayed gland was sufficient to account for the relief of the diabetes. The cause of death was not determined.

In the case of dog 2, which received 4 erythema doses, there was but a trace of sugar in the urine from the 14th to the 19th day following radiation. Occasionally sugar appeared in the urine but never exceeded 5 grams per day. The dog never developed marked symptoms of diabetes. On the 157th day after treatment with x-ray the dog was killed. The portion of the pancreas treated with x-ray had disappeared entirely. At the base of the duct there was sufficient regenerated pancreas to account for the non-development of diabetes.

Dog 3 presents a different picture. This dog was given 4 erythema doses as was dog 2. On the 5th day following radiation the output of urine was increased threefold without an accompanying glycosuria. That is, there was an increase from the normal average daily output of urine of 507 cc. to an average of 1595 cc. This polyuria persisted to the end of the experiment. No glycosuria developed until the 35th day, when a trace of sugar appeared. Complete diabetes developed within one week thereafter. Following this there was a gradual reduction in the sugar excretion during the remaining 2 weeks of life, until there was but a trace of sugar in the urine at the time of death. This animal had all the symptoms of diabetes and was very emaciated at death. The unusual part of the experiment was the early development of the polyuria on the 5th day and the glycosuria on the 35th day. This seems to indicate that the polyuria is not related to a glycosuria or rather to a hyperglycemia. The blood sugar for the first 35 days was within normal limits. The rayed portion of the pancreas

had disappeared but there was sufficient pancreas regenerated from the base of the duct to account for the alleviation of the diabetes. In this case, as in dog 1, death occurred suddenly when the diabetic condition ceased. However, in this case there was a greater degree of emaciation. The liver in both cases did show degenerative changes, but not to as marked a degree as seen in untreated totally depancreatized dogs.

Dog 4 was given 5 erythema doses. Glycosuria appeared on the day following radiation and gradually increased until the 8th day when complete diabetes was attained. There was no recovery of tolerance for carbohydrates, but the usual course of diabetes to extreme emaciation and death. This dog never excreted more than the normal amount of urine in spite of a concentration of sugar as high as 8.8 per cent. The total length of life after radiation was 68 days. From the time that complete intolerance for carbohydrates was attained until death was a period of 57 days. The longest that a dog has been reported to have lived following a total pancreatectomy without treating the diabetes, is 42 days. Dog 4 is a case of complete intolerance for carbohydrates for a period of 57 days. The liver revealed very marked fatty infiltration. Little normal liver tissue was present. Not a trace of pancreas was found at autopsy.

SUMMARY

The cases of dogs 3 and 4 show clearly that polyuria does not always accompany glycosuria. Dog 3 had polyuria without glycosuria, and dog 4 glycosuria without polyuria. When 4 or 5 erythema doses are applied to the pancreas there is complete disappearance of the part rayed with nothing but the pancreatic duct stump to mark the original site of the gland. When 3 erythema doses are applied there is a marked fibrosis of the pancreas and a destruction of islet and acinar tissue. However, in the latter case the rayed portion did not disappear but retained the power of proliferating new pancreas tissue sufficient to relieve the diabetic condition. In spite of the relief of the diabetes in dogs 1 and 3, death occurred without apparent cause.

CONCLUSIONS

1. By means of the above method of isolating the pancreas it is possible to administer known amounts of x-ray to the gland without at the same time affecting other tissues.

2. The application of three erythema doses to the pancreas causes marked fibrosis of the pancreas and diabetes. From this condition the pancreas can recover by proliferating new tissue from the periphery of the gland.

3. Four or five erythema doses applied to the pancreas causes complete disappearance of the gland.

4. Polyuria without glycosuria developed in one dog on the third day following the exposure to four erythema doses.

5. Glycosuria was seen in one dog to the extent of 8.8 per cent without polyuria.

6. One dog lived 57 days with complete intolerance to carbohydrates, which is 15 days longer than the maximum length of life reported for a pancreatectomized dog not receiving insulin.

We wish to express our thanks to Dr. A. J. Carlson for his revision of the manuscript.

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THE FORMATION OF BILE PIGMENT FROM HEMOGLOBIN

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Received for publication December 29, 1925

There is a large amount of data accumulated tending to show that hemoglobin is the source of bilirubin (Rich, 1925). The most important of the proofs submitted are: 1, the chemical relationship between hemoglobin and bilirubin; 2, the formation of bilirubin at localized sites of extravasation of hemoglobin; 3, the increase in the formation of bilirubin following an increase in free hemoglobin in the body.

We have previously proved that bilirubin is formed in the spleen and bone-marrow (Mann, Sheard, Bollman and Baldes, 1925). The knowledge that the formation of bilirubin took place in these two localized areas suggested the possibility of determining whether the injection of hemoglobin would increase the bilirubin production in these two known areas of bilirubin formation.

It is needless to review the very extensive investigation that has been made of the relation of hemoglobin to the formation of bilirubin, as such a task has recently been done by Rich. However, it should be noted that since the publication of the review, Ernst (1925) has reported further studies on the formation of bilirubin following the injection of hemoglobin into the perfused, asphyxiated spleen.

METHOD. All experiments were performed on dogs under ether anesthesia. While the procedures on the major problem only necessitated injecting hemoglobin into, and obtaining blood specimens from two vascular areas, spleen and tibia, it was found, in order to have adequate control data, that several series of experiments were essential. The methods of obtaining the blood specimens and following the rate of bilirubin formation were the same in each series, but they differed in regard to the vascular area which the hemoglobin was made to traverse.

The routine procedure for obtaining blood specimens and injecting hemoglobin, when the spleen was used, was as follows: The organ was exposed through an incision in the median line and wrapped in a moist pack. The vasa brevia and accompanying veins were ligated close to the stomach. A cannula was placed in one of the largest of the ligated

veins with the point of the cannula toward the spleen. The vein was closed with a suitable clamp. Care was taken to have the entering point of the cannula close to one of the large tributaries of the splenic vein. The small artery which accompanied the vein containing the cannula was exposed. A loose ligature was passed around the splenic vein proximal to its formation from the two major branches. The right femoral artery was exposed. The specimen of venous blood (always obtained first) was secured by removing the clamp on the vein containing the cannula and tightening gently the loose ligature on the splenic vein. The obstruction to the splenic vein caused blood to flow backwards through the cannulized vein; this was collected directly in centrifuge tubes. If the valves in the collecting vein hindered the backward flow of the blood too much, they were destroyed by passing a bent wire into the vein and everting them. Very little obstruction to the vein was usually necessary to obtain the specimen. As we have shown that the bilirubin content of the arterial blood is the same in all large arteries, we obtained the samples of arterial blood from the femoral arteries instead of the splenic because of danger of damage to the blood supply to the spleen. The specimens of arterial blood were obtained by direct puncture of the vessel with needle and withdrawal into a syringe. The hemoglobin was slowly injected into the arterial branch accompanying the collecting vein.

While the method employed for obtaining blood specimens from the spleen permitted very satisfactory withdrawal of repeated specimens at definite periods, it did entail a certain amount of exposure and handling of the spleen as well as the production of a slight amount of back pressure in the venous system of the organ. It seemed possible that the vasomotor changes incident to these experimental conditions might squeeze and wash out bilirubin which had been stored in the organ. In order to determine whether these factors were sources of error in our results we performed two series of experiments. In one series the procedures just described were carried out on the spleen and the blood specimens secured at the usual periods of time, but unhemolyzed red cells were injected instead of hemoglobin. These experiments furnished us the opportunity of determining whether or not the procedure followed in obtaining the blood specimen changed the bilirubin content of the blood coming from the spleen. In another series of experiments the entire procedures as first described were carried out. Then the circulation to one small portion, the tip of the tail, was isolated by clamping the organ. As all the venous blood from this portion drained from one branch, it was very easy to obtain all the venous blood that traversed this isolated portion by inserting a cannula into this vein. After specimens of venous blood had been obtained from both portions of the spleen and specimens of arterial blood from the femoral artery, hemoglobin was injected into an arterial side-

branch so that it would traverse the small part of the spleen only, and not enter the general circulation. All the blood which passed through the small portion of the spleen was collected in tubes, and specimens of venous blood were obtained at definite intervals from the major portion of the organ. In these experiments both portions of the spleen were subjected to the same exposure, handling, and so forth, but hemoglobin was injected only into the smaller portion.

The tibia was used for the bone-marrow experiments, and the same method of isolating the circulation to the tibia was used as described in the previous paper. The hemoglobin was injected into the arterial branch nearest the main artery going to the tibia. The arterial specimens were obtained from the opposite femoral artery.

It was found, as the isolation of the circulation to the tibia necessitated so much operative trauma and consumed so much time, that, after the control specimens of blood were obtained, the circulation through the tibia had become so reduced that little effect could be expected from the injection of hemoglobin. As we had previously shown that the increase in the bilirubin content of the femoral vein over that in the femoral artery is due to the addition of bilirubin to the blood passing through the tibia, it appeared sufficient, in order to determine whether the injection of hemoglobin would increase the amount of bilirubin formed in the marrow, to inject the hemoglobin directly into the femoral artery. Accordingly, in a few experiments the circulation of the tibia was not isolated but the hemoglobin was injected into the femoral artery, the specimens of venous blood being obtained from the femoral vein of the same side and the specimens of arterial blood from the femoral artery of the opposite side.

For further control experiments, the gastrocnemius muscle was isolated as described in our previous paper, hemoglobin injected into the arterial stream entering it, and the specimens of venous blood obtained directly from the muscle. Finally in a small series of experiments, the hemoglobin was injected into the jugular vein and the specimens of venous blood obtained from the various large veins of the body at various periods after operation.

In all experiments a blood specimen was obtained from the vein and then the artery. Hemoglobin was then slowly injected and blood specimens from both the vein and artery secured at definite intervals of time.

Hemoglobin solutions for injection were prepared from freshly drawn blood of normal dogs. The blood was withdrawn from the jugular vein into a glass syringe containing sodium citrate sufficient to make approximately 1 per cent of the blood mixture. The blood was then centrifuged at high speed and the cells washed twice with physiologic solution of sodium chlorid. After the removal of the supernatant liquid from the second washing, sufficient distilled water was added to the cells to

make the volume equal to that of the blood taken. This mixture was shaken and allowed to stand for five minutes before the addition of the small amount of sodium chlorid necessary to make the solution isotonic with blood.

The technic for preparing the solution for examination in the spectro-

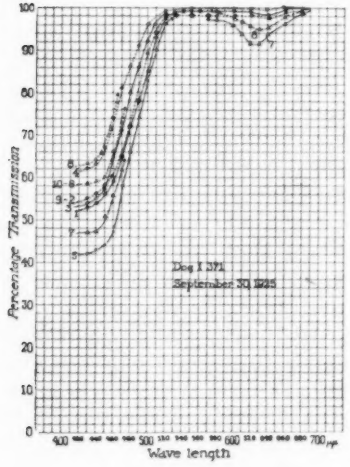

Fig. 1

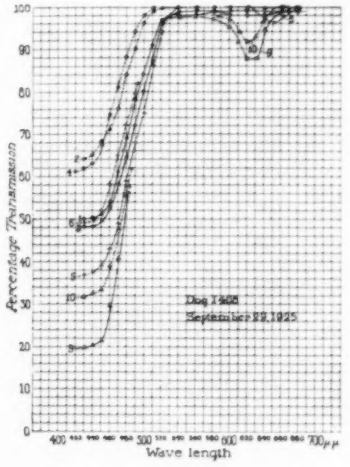

Fig. 2

Fig. 1. Effect of the injection of hemoglobin on the concentration of bilirubin in blood from the splenic vein. The venous blood for curves *1* and *3*, and the arterial blood for curves *2* and *4* were taken before the injection of 2.5 cc. of laked blood. Blood for curves *1* and *2* was withdrawn ½ hour before that for curves *3* and *4*. For curves *5*, *7* and *9* venous blood, and for curves *6*, *8* and *10* arterial blood was taken at intervals of 30 minutes, 1 hour and 1½ hours after the injection. Curves *5* and *7* show the increase in bilirubin in the venous blood ½ hour and 1 hour after the injection of hemoglobin. Only slight change in the bilirubin content is shown by any other curve. The absorption at 620 to 640mμ (hematin) accompanies the increase in the production of bilirubin.

Fig. 2. Effect of the injection of hemoglobin on the concentration of bilirubin in blood from the splenic vein and in arterial blood. Curves *1*, *3*, *5* and *9* are for plasma from the splenic vein just before, and at intervals of 15 minutes, 30 minutes and 2 hours after injection of 2.5 cc. of laked blood into the splenic artery. Curves *2*, *4*, *6* and *8* are for plasma from the femoral artery just before, and at intervals of 15 minutes, 30 minutes and 2 hours after the injection of the laked blood. Increase in both arterial and venous bilirubin is shown.

photometer as well as the method of employment of this instrument was the same as that given in the previous paper. It should be again emphasized that conclusive results in regard to either the presence or the amount of bilirubin in a solution cannot be obtained if the solution is turbid or

contains hemoglobin. As the amount of hemoglobin injected in these experiments was small, and as considerable time had elapsed after injection before the samples of blood were collected, the injected hemoglobin did not cause any serious difficulties.

RESULTS OF EXPERIMENTS. The results of the experiments can best be presented in relation to the different types of experiments performed.

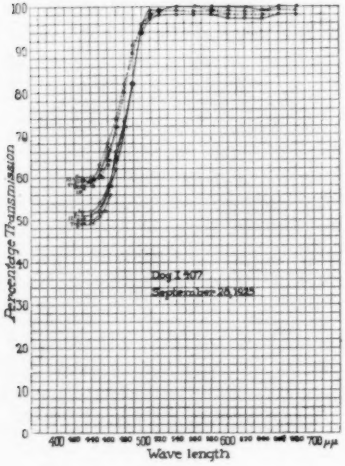

Fig. 3

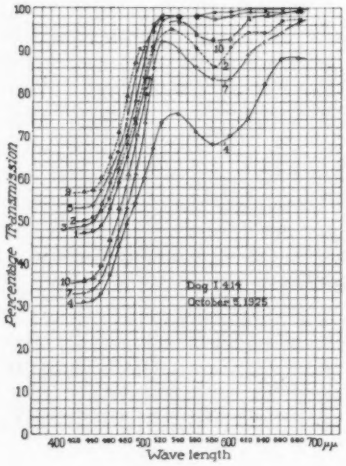

Fig. 4

Fig. 3. Effect of the injection of red cells on the concentration of bilirubin. Curves *1*, *3*, *5*, *7*, *9* and *11* show transmission of light through plasma taken from the splenic vein at 30-minute intervals. Curves *2*, *4*, *6*, *8*, *10* and *12* are for blood from femoral artery at 30-minute intervals. Immediately after withdrawal of blood for curves *3* and *4*, washed red cells in 5 cc. of physiologic solution of sodium chlorid was injected into the splenic artery. The bilirubin concentration in the splenic vein is consistently greater than in the arterial blood and not affected by the injection of red cells.

Fig. 4. Comparative results of injection of hemoglobin into the isolated portion of spleen. Curves *3*, *4*, *7* and *10* are for venous plasma from isolated portion before and at intervals of 15 minutes, 1 hour and 2 hours after injection of 2.5 cc. of laked blood into its artery. Curves *1* and *8* are for the venous plasma from the uninjected portion of the spleen before, and one hour after, the injection of the other portion. Curves *2* and *9* are for plasma from the femoral artery, taken at the same time as that from the unisolated portion of the spleen. The blood from the uninjected portion showed no increase in bilirubin.

Experiments on the spleen. The venous blood secured from the splenic vein always had a higher content of bilirubin than the arterial blood. In some animals this difference was very marked while in others it was scarcely measurable. The injection of hemoglobin into the blood entering the spleen was, with few exceptions, followed by an increase in the bilirubin

content of the blood of the splenic vein over the amount found before injection (fig. 1). Again there was considerable variation in the results in the different animals. In some experiments the increase in the bilirubin content following injection was very great while in other experiments it was very slight, and rarely there was none. In a very few experiments this increase in the bilirubin content of the venous blood was measurable at the end of fifteen minutes after the completion of the injection of the hemoglobin, but usually the increase was not evident before thirty minutes. The bilirubin content usually continued to increase for one or two hours after injection, and then decreased. In some experiments the bilirubin content of the arterial blood also increased greatly after the injection of hemoglobin into the circulation of the spleen (fig. 2). In the experiments in which unhemolyzed red cells instead of hemoglobin were injected into the blood going to the spleen, the other procedures being the same as when hemoglobin was injected, no increase in the bilirubin content of the blood of the splenic vein above the preinjection amount occurred (fig. 3). The experiments dealing with the injection of hemoglobin into an isolated portion of the spleen were difficult to perform and there were several failures, due to the decrease or complete stoppage of the circulation through the isolated portion following the injection of the hemoglobin. In order to prevent the animal from losing too much blood during the period of the experiment, it was necessary to isolate only a very small area. The injection of the hemoglobin into the small vascular areas was often followed by an increase in coagulability of the blood which would prevent the withdrawal of blood for a considerable time after injection. However, by using extreme care in avoiding trauma at the time

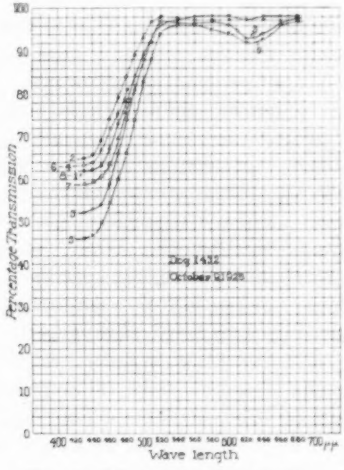

Fig. 5. Effect of the injection of hemoglobin into the general circulation on the concentration of bilirubin in blood from the splenic vein. Blood for curves *1* and *2* was taken before the injection of hemoglobin. The curves with odd numbers represent the bilirubin content in blood from the splenic vein. The curves with even numbers represent the bilirubin content of blood from the femoral artery. Laked blood was injected into the jugular vein. Subsequent withdrawals were made at intervals of one, two and three hours. The increase in bilirubin is shown in curves *3* and *5*, and there is some absorption at 620 to 640mμ (hematin) in these two curves.

of injection, we were able to maintain an adequate circulation in a few experiments. In these successful experiments the bilirubin content of the blood coming from the isolated portion of the spleen into which the hemoglobin had been injected, increased over its pre-injection level, but remained practically the same throughout the period of the experiment in the blood coming from the major portion of the spleen into which hemoglobin was not injected (figs. 4 and 5).

Experiments on bone-marrow. The experiments dealing with the injection of hemoglobin directly into the blood going to the tibia were difficult and the results, in general, inconclusive. However, suggestive evidence was secured that such injections were followed by an increase in the bilirubin content of the blood returning from the tibia. The bilirubin content of the blood in the femoral vein was always higher than that of the femoral artery. When the hemoglobin was injected into the femoral artery and the venous specimen obtained from the femoral vein, an increase in the bilirubin content of the blood of the femoral vein over the pre-injection level always occurred.

Experiments on muscle. When hemoglobin was injected into the blood going to muscle only, no increase in bilirubin occurred and, furthermore, the bilirubin content of the venous blood returning from muscle and that of the arterial blood were the same.

Experiments on general circulation. The injection of hemoglobin directly into the systemic venous system (jugular vein) and withdrawal of blood samples from the same vessel, showed a very slight increase in bilirubin. This was much less than when the hemoglobin was injected directly into the splenic circulation and the specimen secured from the splenic vein.

Nature of spectrum. In the analysis of the various curves it was readily noticed that, as the formation of bilirubin increased after the injection of hemoglobin, the character of the curves changed in regard to an absorption of the light in the region of 590 to 660 mμ, with maximal absorption of 620 to 640 mμ. The amount of this absorption increased in general as the rate of bilirubin increased, and then decreased as the rate of bilirubin decreased until it disappeared when the bilirubin content approached the pre-injection level. This change of the character of the curve clearly indicated that a substance other than hemoglobin or bilirubin was being formed which reached its maximal value simultaneously with that of bilirubin.

Discussion. The results of the experiments, while subject to considerable variations quantitatively, were very definite in three respects: 1, there was always a larger amount of bilirubin in the blood returning from the spleen and tibia than in the arterial blood; 2, a definite increase in the amount of bilirubin returning from the two areas was produced by

the injection of hemoglobin; 3, a substance other than hemoglobin or bilirubin appeared during the time the bilirubin content was increasing after the injection of hemoglobin, and then disappeared as the bilirubin content decreased.

The results of these experiments have greatly augmented the data presented in the previous paper in regard to bilirubin being formed in the spleen and bone-marrow. In all the experiments in this investigation, it was necessary to estimate both the bilirubin content of the venous blood draining from the vascular area to be subsequently subjected to the injection of hemoglobin and that of the arterial blood. In all the experiments in which it has been possible to make a determination, the bilirubin content of the blood draining from the spleen and marrow has been greater than the bilirubin content of the arterial blood. It should again be emphasized that we have always found that the bilirubin content of the blood obtained from the various arteries is the same. While there was always a difference between the bilirubin content of the venous blood of the spleen and tibia and that of the arterial blood, this difference was subject to very great variation in the different animals. In some animals it was relatively very great while in other animals it was hardly measurable.

In most of the experiments the injection of hemoglobin directly into the blood entering the spleen, produced an increase in the bilirubin content of the blood leaving the spleen above the pre-injection level. In a few experiments the increase in bilirubin following injection of hemoglobin was insignificant. No definite reason for this difference was found. While there was a definite and measurable increase in the bilirubin content of the splenic vein following the injection of hemoglobin into the blood entering the spleen in most of the experiments, it was found impossible to compare the results of the experiments quantitatively. In some animals the increase in bilirubin was very marked while in other animals it was hardly measurable. However, considering the investigation as a whole, sufficient positive evidence was obtained to justify fully the conclusion that bilirubin is formed from hemoglobin in the spleen and marrow. The control experiments, particularly those in which unhemolyzed cells were injected instead of hemoglobin and those in which a small portion of the spleen was isolated, conclusively proved that the increase in bilirubin in the splenic vein following the injection of hemoglobin was due to the hemoglobin injection and not to the technical and operative procedure employed.

Very early in these experiments in which hemoglobin was injected, it became evident that some substance other than hemoglobin or bilirubin was present in the blood withdrawn during the period of the formation of bilirubin from hemoglobin. After the hemoglobin injections, a new absorption band appeared in the spectrum of the blood subsequently with-

drawn, so that at the time of increase of formation of bilirubin from hemoglobin, the spectrum showed an absorption band with maximal value at 620 to 640 mμ which was not present before injection and disappeared when the increase in bilirubin subsided. A value of 630 to 640 mμ is given as the characteristic absorption spectrum of hematin.[1] Other substances which have absorption bands near this spectral region do not seem likely steps in the formation of bilirubin from hemoglobin. There is a large amount of evidence which would indicate that hematin is formed as an intermediate product in the breaking down of hemoglobin.

The appearance of hematin during the active formation of bilirubin would indicate that the process of disintegration of hemoglobin into hematin and globin occurs more rapidly than the formation of bilirubin from hematin. Thus the presence of hematin in the blood in disease might easily be interpreted as evidence of increased destruction of hemoglobin. This finding would apparently be of considerable importance in the study of hemolytic icterus and also of value in differentiating the various types of anemia.

In some experiments, not only was the injection of hemoglobin followed by an increase in the bilirubin content of the blood of the splenic vein above the pre-injection level but there also was a gradual increase in the bilirubin content of the arterial blood. . This could have been due to one or both of two causes. Much of the hemoglobin injected into the blood going to the spleen must have passed through that organ and entered the general circulation. Other bilirubin forming cells probably converted this readily available hemoglobin into bilirubin and caused an increase in the bilirubin content of the blood over the pre-injection level. Again the liver does not secrete bile at the normal rate when an animal is under an anesthetic. A part of the increase in the bilirubin content of the blood might have been due to a decrease in the rate of elimination. While both of these factors were possibly responsible for the gradual increase in the bilirubin content of the arterial blood following the injection of hemoglobin into the splenic circulation, the first factor was the most important because there was usually a gradual decrease in the bilirubin content of the arterial blood toward the end of the experiment, one and a half or two hours after the injection of the hemoglobin.

[1] Allen, Commercial organic analysis, xiii, gives for hematin in alcoholic or acetone solution 630 mμ with very faint bands at 578 and 540 mμ. The latter would not show in our work. Kayser, H.: Handbuch der Spectroscopie, iv, p. 126: Alcalische Hb zeige ein Band mit der Mitte bei 618, ihr neuer Körper ein solches bei 656. On page 127 is a series of data such as: free iron (Preyer) 630–626; in alcohol (Gamgee) 636–618, (Jäderholm) 650–630; in ether (Halliburton) 656–615, (Ziemke and Müller) 644–634 mμ.

SUMMARY

Previous work has proved that bilirubin is formed in the spleen and bone-marrow. The present investigation was undertaken for the purpose of determining whether the injection of hemoglobin into the blood entering these two known areas of bilirubin formation would be followed by an increase in the amount of bilirubin produced. The results of the experiments conclusively proved that the injection of hemoglobin into the blood entering the spleen and marrow does produce an increase in the amount of bilirubin formed at these sites. Furthermore, it was found that, at the time that the bilirubin increased following the injection of hemoglobin, another substance appeared. This substance appears to be hematin. Adequate control experiments were performed to eliminate sources of error completely in either obtaining or interpreting the data. Our investigations to date have proved the following: 1, bilirubin is formed in the spleen and marrow; 2, bilirubin is formed from hemoglobin; 3, hematin appears as an intermediate product in the formation of bilirubin from hemoglobin.

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HYDROGENATED VEGETABLE OIL AS A SOURCE OF VITAMIN E[1]

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Received for publication January 7, 1926

In 1923 we had occasion to use a modification of Evans and Bishop's Standard Diet II (1) in connection with an extensive study we were making on growth and reproduction. While this special experiment did not give the information we were looking for, it did uncover an unexpected source of vitamin E, namely, in a commercially hydrogenated cotton seed oil, Crisco. Evans and Burr (2) having recently arrived at the same result independently, its correctness would seem to be definitely established.

As pointed out by Evans and Bishop, animals are usually sterile when reared on their Standard Diet II which consists of casein 18, cornstarch 54, lard 15, milk fat 9, salts 4, and 0.4 to 0.6 gram dried yeast daily. A second generation was occasionally secured which was invariably sterile. These investigators point out that this ration was not in any sense highly purified as the casein used was merely a high grade commercial product. This was also true of the cornstarch used.

As our investigation did not have the same purpose as that of Evans and Bishop we modified the above ration in the following ways:

Rations 34 and 36		Rations 35 and 37	
Casein (purified)[2]	18	Casein (purified)[2]	18
Starch	54	Dextrin (purified)	54
Crisco	15	Crisco	15
Milk fat	9	Milk fat	9
Salts	4	Salts	4

With each of these rations 0.6 gram dried yeast was given daily.

These experiments were carried out in the following manner: Five or

[1] Published with the approval of the Director as Paper no. 595, Journal Series, Minnesota Agricultural Experiment Station.

[2] In ration 34 the casein was precipitated from skim milk by HCl reprecipitated twice from ammonium hydroxide solution and finally leached for 7 days with daily changes of water at a pH 4.8. In rations 35 and 36 the casein was purified according to a method used in this laboratory which will be given in a later paper. In ration 37 commercial casein was purified by the method prescribed by McCollum and Davis (3). The dextrin was purified by alcohol and ether extraction.

more young rats four or five weeks old were kept in colonies in laboratory cages which have a floor of three mesh wire netting raised one and one-half inches from the sawdust covering the metal pan below the screen. The food was weighed into a tin container which prevents scattering of the food and thus makes possible a fairly quantitative record of the food intake. The rats were weighed once a week. Pregnant females were isolated before the birth of their young. The rations were made according to the method described by Evans and Bishop (1). The yeast was a pure powdered product manufactured by the Northwestern Yeast Company and fed separately in tablet form.

Table 1 is a brief summary of the experiment. The growth curves of

TABLE 1

Number of animals, duration of experiment and reproduction record of females on standard diets containing 15 per cent Crisco

NUMBER OF RATION	NUMBER OF RATS IN						TIME OF EXPERIMENT	TOTAL NUMBER OF LITTERS	NUMBER OF LITTERS WEANED
	First generation		Second generation		Third generation				
	♂	♀	♂	♀	♂	♀			
							months		
34	2	3					8.0	10	1
35	1	2					4.5	6	2
35			2	4			5.0	7	1
35					1	1	4.5	1	0
36	2	5					4.5	6	2
36			1	4			4.5	8	2
36					2	3	5.5	3	0
37	2	2					7.5	7	2
37			3	3			5.0	5	2
37					3	7	7.0	15	2*

* One male rat in each of 2 litters reared.

the females whose reproduction records are given are shown in figure 1.

It can be readily seen from both the table and the chart that the mother rats had great difficulty in rearing their litters. Following several failures of this character early in the experiment we considered that this might be due to a deficiency or lack of some lactation factor in the diet. Thereafter as soon as the young were born a daily supplement of 15 cc. of whole milk was given to each mother and continued throughout the lactation period. However, this amount of milk when used as a supplement to the ration did not prove to be entirely adequate as a carrier of such a factor. Out of a total of 68 litters 13 may be said to have been successfully reared to weaning. In two other litters one rat only in each litter was reared. If the 16 litters which were eaten by the mother are deducted from the total,

it is seen that 25 per cent of the rem ining litters was successfully reared to weaning.

From an inspection of figure 1 it is seen that throughout the experiment the rate of growth was very good and that in the majority of cases reproduction was very satisfactory. Furthermore, we could have doubtless carried the experiment through more generations had it not been for the difficulty the mother rat had in rearing her young.

The explanation of the reproduction activity of the females in our experiment far beyond the period when sterility should have occurred according

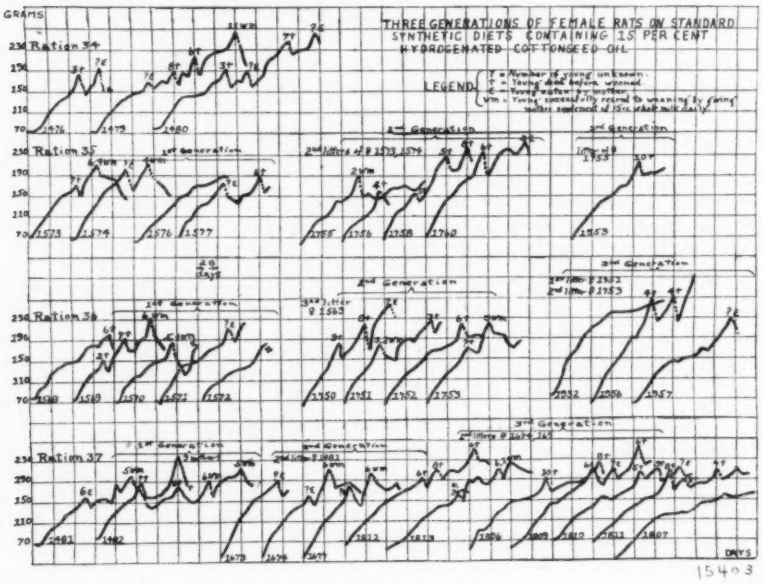

Fig. 1

to Evans and Bishop's results seems to be explained only by the fact that we employed Crisco in the basal diet in place of the lard which Evans and Bishop used. Whether the ovulation rhythm was more regular on our rations than on Evans and Bishop's is not known as we used only the gross means of detecting reproduction, that is, birth of young. However, it is quite evident from the behavior of our rats that placental function was stimulated by a factor carried by the Crisco.

At first sight it appears that reproduction was better on the rations containing the less highly purified casein, namely, rations 34 and 37, but this is not borne out when the results are reduced to a uniform time basis.

SUMMARY

We have shown that successful reproduction can be secured in three successive generations of rats on the Evans and Bishop sterility diet when lard is replaced by hydrogenated vegetable oil. It may be inferred from these observations that hydrogenation does not destroy the fertility factor, vitamin E.

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THE ELECTRIC CAPACITY OF ANIMAL TISSUES UNDER NORMAL AND PATHOLOGICAL CONDITIONS[1]

Preliminary Report

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Received for publication January 8, 1926

This research which has been recently initiated in the Biophysics Laboratories of the Cleveland Clinic Foundation is a logical sequence of researches already reported in which an attempt has been made to secure by means of physical measurements further information regarding the organs and tissues primarily concerned in the operation of the animal organism as a whole. The initial biophysical studies were measurements of the electric conductivity of tissues under normal and pathological conditions. This research was followed by measurements of variations in the temperature in the living animal by means of especially constructed thermocouples.

Following the above studies formulae for capacity measurements of animal tissues were devised by Doctor Fricke and measurements were made by means of apparatus devised by him by means of which capacity measurements can be made at frequencies varying from eight hundred to four and one-half million cycles (1). Doctor Fricke has previously reported his measurements and calculations of the capacities of suspensions of red corpuscles (2) from which he derived the thickness of the corpuscle membrane, and Doctor Fricke and Doctor Morse have reported at a recent meeting of the Society of Cancer Research their measurements of the capacities of malignant and benign tumors (3). The apparatus devised by Doctor Fricke and his formulae are being used in the present work and we wish to express our appreciation of his suggestions and aid. The first measurements in this study were made by George H. Crile; the measurements now in progress are being carried on by Miss Lillian Jacobson.

Thus far we have been searching for leads to determine what should be the trend of our later investigations, and we are not prepared to offer any interpretation of these preliminary results. They are presented at this time, however, because we have already accumulated sufficient

[1] Read before the American Physiological Society, Cleveland, December 29, 1925.

data to indicate that in common with conductivity and temperature measurements, capacity measurements will probably throw valuable light upon certain physiological problems.

Forty-one animals have been used in these preliminary experiments. As stated above, the measurements have been made by the specially designed bridge described by Doctor Fricke in his paper on the electric capacity of suspensions of red corpuscles of a dog (2). The frequency used has been 20,000 cycles. The bridge may be briefly described as follows: two arms contain a Kohlrausch slide-wire which is always used near its middle point; the third arm contains a decade resistance box with a decade condenser in parallel; and the fourth arm, the electrolytic cell with a variable condenser and resistance in parallel. By means of a switch the cell can be replaced by a decade resistance box and the variable condenser. The current to the bridge is supplied by an audion oscillator and a heterodyne method of detection is employed, using a three stage amplification. The bridge is connected to the generating and the heterodyne oscillator and to the detector tube by a very loose inductive coupling. The electrolytic cell is made of glass and has two movable parts which are symmetrical and between which a diaphragm containing the tissue is placed. The cell is then filled with Ringer-Locke solution.

Various checks and controls have been employed. The room temperature has been kept at about 20°C. The pieces are cut as uniformly as possible and not less than four specimens are used in determining the average for the liver of each animal. Measurements are made at as uniform a time after death for both normals and abnormals as is possible; 1 hour for the liver; 2 hours and 20 minutes for the cerebrum; $2\frac{3}{4}$ hours for the cerebellum; and $3\frac{1}{4}$ hours for the medulla oblongata. The differences in capacity due to the time factor are much less in different animals than in specimens of the same organ from the same animal. Several experiments have been carried out to determine the change of capacity with time, which have shown that the capacity of the liver and brain decreases gradually with time.

The capacity range of normal tissues with large enough percentages of error to account for the differences between animals of different sizes and ages is shown in table 1. It is also interesting to note that although the capacities of nerves are not included in the table, a few measurements have shown that they are higher than the capacity of the brain.

A group of 14 animals of like size and in most instances of the same breed were used in the following experiments. Measurements of normal animals were distributed among those of abnormal animals so as to avoid changes due to the confinement of the animals, one normal rabbit being measured at the beginning, one in the middle and one at the end of the series.

TABLE 1

Preliminary measurements; capacity range of normal tissues

TISSUE	C_S IN M.M.F.	ERROR
		per cent
Voluntary muscle (leg)	4600	10
Kidney	3800	10
Liver	3700	5
Adrenal	1870	10
Heart	1300	15
Lung	1210	10
Cerebrum	1020	4
Cerebellum	1077	7
Medulla oblongata	1000	8
Stomach muscle	900	15
Spleen	800	20
Spinal cord	650	15
Fascia of leg muscle	200	15
Fat	70	10

TABLE 2

Capacity measurements; preliminary

TISSUE	NUMBER OF ANIMALS	CONDITION	MEAN C_S M.M.F.	ERROR OF MEAN
				per cent
Liver	3	Normal	3674	4.05
Liver	3	HCl	3214	3.12
Liver	4	Adrenalin	3580	3.1
Liver	3	$NaHCO_3$	2513	9.0
Cerebrum	3	Normal	1024	3.66
Cerebrum	3	HCl	963	3.6
Cerebrum	4	Adrenalin	916	2.29 (% av.)
Cerebrum	1	Hepatectomy	1077	(5.25)
Cerebrum	3	$NaHCO_3$	902	0.32
Cerebellum	3	Normal	1077	6.6
Cerebellum	3	HCl	1127	1.6
Cerebellum	4	Adrenalin	1039	3.6 (% av.)
Cerebellum	1	Hepatectomy	1080	(3.0)
Cerebellum	3	$NaHCO_3$	1025	2.88
Medulla oblongata	3	Normal	997	6.15
Medulla oblongata	3	HCl	922	1.25
Medulla oblongata	4	Adrenalin	929	6.3 (% av.)
Medulla oblongata	1	Hepatectomy	1005	(0.35)
Medulla oblongata	3	$NaHCO_3$	837	5.5

The results of this series as shown in table 2 may be briefly summarized as follows:

The indications are that the injection of *hydrochloric acid* decreases the capacity of the cerebrum, the medulla oblongata and the liver, although the changes are not definite enough to fall outside the percentage of error.

The effects of *adrenalin* are uncertain.

Hepatectomy seems to increase the capacity of the cerebrum, cerebellum and medulla oblongata.

The indications are that the injection of an *alkali*—sodium bicarbonate—decreases the capacity of the cerebellum and the liver. In the experiments with sodium bicarbonate it is interesting to note that the decrease

TABLE 3

Effect of alkali on C_s *liver*

DOSE OF ALKALI	TIME KILLED	C_s
cc.		
20	15 minutes later	3070
25	2 hours later	2540
20		
10*	1 hour later	1930

* Three hours after first dose.

TABLE 4

Effect of injection of ether on C_s

TISSUE	NORMAL	ETHER
Liver	4250	1940
Brain	1100	800
Kidney	3500	2000
Spinal cord	700	270

in the capacity of the liver is in direct relation to the dose of the alkali. In one animal, 20 cc. of saturated sodium bicarbonate was injected into the ear vein. The animal was killed in 15 minutes. Its liver capacity was 3,070. Another animal was given 25 cc. of saturated solution of sodium bicarbonate and was killed two hours later. Its liver capacity was 2,540. A third animal was given one dose of 20 cc. saturated sodium bicarbonate and three hours later another dose of 10 cc., being killed one hour after the last dose. The capacity of the liver of this animal was the lowest, only 1,930. (See table 3.)

The effect of the injection of ether directly into the tissues was a marked decrease in the capacity of each of the tissues measured—liver, brain, spinal cord and kidney. (See table 4.)

There seems to be a gradual increase in capacity with the age and size of the animals. In rabbit fetuses the capacity of the liver was about 1800 m.m.f., while the capacity of the liver of the mother was 3,300; and the capacity of the liver of one rabbit which was much larger and sturdier and apparently older than any other in the group was 4,800.

SUMMARY

Preliminary measurements of the capacity of certain organs and tissues of rabbits, of the brain and liver in particular, have indicated but have not as yet proven that in common with electric conductivity and temperature the capacity of a tissue is also a constant which is susceptible to change under varying conditions.

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DIET IN RELATION TO REPRODUCTION AND REARING OF YOUNG

I. Observations on the Existence of Vitamin E

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Received for publication January 9, 1926

During the past few years considerable work has been done on the existence of an unknown dietary factor for reproduction. Evans (1922) and associates at the University of California were the first to bring forth this idea that the known dietary factors for normal growth are insufficient for reproduction. They maintain that another unknown principle, vitamin X, is required for the normal production of young. Sure (1923) has also contributed several articles to the solution of this problem and has studied its distribution in various naturally occurring food materials. He designates this unknown chemical factor vitamin E, which nomenclature has been recently adopted by Evans and co-workers. Mattill and Stone (1923) have likewise presented evidence that another factor in addition to those already known is required for normal functioning of the reproduction process. It is impossible in a short article such as this to present all of the evidence in support of the existence of this unknown chemical complex and so only the work bearing on the problem at hand will be considered.

Evans and Bishop (1922) claim that on synthetic rations containing yeast as the sole source of vitamin B and filtered butterfat as the only source of vitamin A rats fail to reproduce, although they grow to full adult size at the normal rate. These investigators employed a ration which had the following composition: Casein 18 per cent, cornstarch 54 per cent, lard 15 per cent, butterfat 9 per cent, salts 4 per cent, and yeast 0.4 gram daily. Kennedy and Palmer (1922) likewise employed purified diets, containing yeast as the sole source of vitamin B, which were supposedly sufficient for the needs of the animal body, but few of the animals reproduced. The conclusion of these workers was that yeast is not a very rich source of vitamin B. This idea seemed contrary to the experiences of most investigators for repeated experiments seemed to indicate that yeast was one of the most potent sources of this particular vitamin. Heller (1923) of this laboratory demonstrated at about the same time that yeast contains an abundance of vitamin B. In one of a series of articles from

this laboratory by Nelson, Heller and Fulmer (1923) it was shown that it is not necessary to assume the existence of a new vitamin for reproduction. Rats receiving a synthetic ration in which yeast supplied the only source of vitamin B reproduced on levels of yeast as low as 1.5 per cent. The surprising observation was made, however, that most of the young were not reared on the different levels of yeast employed. Third generation animals were weaned when the diet contained 5 per cent of yeast as the only source of vitamin B, but not all of the young were reared. The young which were not reared on the various yeast levels died in an emaciated condition in a period of time varying from 1 to about 4 weeks. Although growth in the first generation was normal or better than normal, the young did not behave in a normal manner even though they were normal in weight at birth and, as far as could be ascertained, perfectly healthy. The idea prevailed that insofar as the mortality of the young was concerned, something was wrong as regards the physiology of milk secretion resulting in a diminished flow or altered composition of the milk or both.

Other experiments of a different kind seemed to point to the conclusion that another vitamin was necessary for reproduction. Mattill and Stone (1923) stated that on diets in which all of the protein and vitamins were given in the form of dry milk, rats grew at the normal rate up to 75 days of age and that subsequently the rate fell below normal, the decline being more marked in females than in males. At 175 days of age the females were 14 per cent underweight and the males were 5 per cent below the average weight. Reproduction was unsuccessful on all of the diets containing milk powder which were used. These authors suggest that the reproductive failure on milk rations might be due to a lack of the unknown dietary factor known as vitamin E, which Evans and Bishop (1922) have shown is essential for normal reproduction. Anderegg (1924) of this laboratory has done extensive work on the nutritive value of milk, and his conclusions may be summarized as follows: *1.* Normal growth and reproduction result when whole milk powder is the only source of protein and vitamins in the diet. *2.* The mortality of the young on many of the diets recorded is high. *3.* In order to obtain optimum results in nutrition the proportion of fat, protein, and inorganic constituents must be within certain limits. *4.* The proportion of protein in the diet has a marked influence on the well-being of the suckling although the first generation may grow at the normal rate. *5.* Data show that it is unnecessary to assume the existence of a new vitamin for reproduction. Subsequent work from this laboratory by Anderegg and Nelson (1925) demonstrates that whole and skimmed milk powders have entirely different food values aside from the difference in fat content. Whole milk powder properly supplemented with iron salts and carbohydrate or iron salts alone furnishes everything necessary for growth, reproduction, and rearing of young. It was found that the relative

amounts of protein, fat and salts markedly influence the nutritive value of the diet. In particular the quantity of iron salts added had a pronounced effect on reproduction. On skimmed milk powder diets of the same composition as whole milk powder diets, which gave favorable results on reproduction, rats failed to reproduce normally, although they grew at the normal rate.

Evidence is by no means conclusive for the existence of vitamin E. We have been aware for a long time that a big problem exists in reproduction and were engaged in this field of investigation before other investigators proposed the existence of an unknown chemical complex which is required for this physiological process. In the course of our investigations we employed the diet which had been observed by Evans and Bishop to fail during the period of reproduction. Osborne and Mendel (1919) have employed similar diets and noticed that although the animals grew vigorously to full adult size nevertheless they were, with very few exceptions, found to be sterile. We found that reproduction was not normal on this diet, but by slight modification of the amounts of the individual dietary constituents of this ration we kept animals going through three generations. Our first impression was that of Evans and Bishop, that perhaps an unknown factor was necessary for reproduction, but due to the fact that some of the animals reproduced repeatedly and the fact that we obtained several generations, this view appeared untenable, or at least we felt that this idea should be postponed until more conclusive evidence had been established. The conclusion of a new vitamin for reproduction might appear warranted from the results obtained in this laboratory by Anderegg and Nelson (1925) on whole and skimmed milk powders. The fact that skimmed milk powder diets of the same chemical composition as whole milk powder diets, on which good reproduction records were obtained, failed during the period of reproduction, although growth was normal, would seem to indicate that in the manufacture of butter some unknown substance which is essential for reproduction was removed or destroyed. However, additional unpublished data have been obtained by Anderegg and Nelson which may throw a different interpretation on the problem. It has been found that if cod liver oil is fed separately from the other ingredients of the skimmed milk powder rations, then the animals reproduce, whereas, if incorporated in the dry ration, young are not born. Fourth generation young have been reared on skimmed milk powder diets moistened with water and with cod liver oil fed separately. Cod liver oil undergoes decomposition when included in certain rations with the production of substances having an odor resembling that of acrolein. Water retards this decomposition. Now cod liver oil has been shown by Evans and Bishop (1923) and Sure (1923) not to contain vitamin E. Yet these experiments show that on skimmed milk powder diets with cod liver oil

as the only source of fat soluble vitamins animals have been carried through the fourth generation.

One thing is certain, that reproduction is not normal on certain synthetic diets or certain diets involving restricted food materials. On such diets the number of young born may be comparatively few, and the vast majority of the young die before they are weaned, although it has been possible

TABLE 1

Composition of diets

DIET NUMBER	CASEIN	SALTS	BUTTERFAT	YEAST	DEXTRIN
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	18	3.7	5	1.0	72.3
2	18	3.7	5	1.5	71.8
3	18	3.7	5	2.0	71.3
4	18	3.7	5	2.5	70.8
5	18	3.7	5	3.0	70.3
6	18	3.7	5	3.5	69.8
7	18	3.7	5	5.0	68.3
8	18	3.7	5	8.0	65.3
9	18	3.7	5	12.0	61.3
10	18	3.7	5	16.0	57.3

TABLE 1A

Reproduction records obtained on diets given in table 1

DIET NUMBER	NUMBER OF MALES	NUMBER OF FEMALES	NUMBER OF LITTERS	TOTAL YOUNG	YOUNG REARED	YOUNG DIED	YOUNG CONSUMED	YOUNG DIED WITH MOTHER	CURVE OF GROWTH
1	4	7	0	0	0	0	0	0	−
2	3	7	2	12	0	6	6	0	−
3	1	4	4	26	0	12	14	0	+
4	10	14	11	59	15	35	0	9	+
5	17	23	21	129	28	88	0	13	++
6	6	10	8	54	14	18	22	0	++
7	36	64	58	357	233	91	13	12	+++
8	6	10	15	93	51	28	14	0	+++
9	3	3	8	61	52	0	9	0	+++
10	2	4	5	34	22	1	11	0	+++

to carry animals through several generations on these diets. It is possible that an unknown factor of the nature of a vitamin is necessary for milk secretion and as a matter of fact such interpretations have appeared in the literature (Sugiura and Benedict, 1919). We had that idea long ago but just as we felt it premature to assume the existence of a new vitamin for reproduction so we believed there was not sufficient data for the assumption of the other vitamin. We do not deny both of these vitamins may exist, but data are not sufficiently conclusive for their existence. On our

breeding ration which consists of a mixture of naturally occurring food materials rats are very prolific and practically all of the young are reared. Why is it that on naturally occurring food mixtures such favorable results are obtained, whereas on synthetic diets, supposedly complete, failure

TABLE 2

Composition of lard diets

DIET NUMBER	CASEIN	SALTS	BUTTERFAT	LARD	YEAST	DEXTRIN
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	18	3.7	5	7	5	61.3
2	18	3.7	5	15	5	53.3
3	18	3.7	5	19	5	49.3
4	18	3.7	5	25	5	43.3

TABLE 2A

Reproduction records on diets in table 2

DIET NUMBER	NUMBER OF MALES	NUMBER OF FEMALES	NUMBER OF LITTERS	TOTAL YOUNG	YOUNG REARED	YOUNG DIED	YOUNG CONSUMED	CURVE OF GROWTH
1	5	8	0	0	0	0	0	+++
2	3	7	0	0	0	0	0	+++
3	4	12	1	8	8	0	0	+++
4	3	6	0	0	0	0	0	++

TABLE 3

Composition of lard and high casein diets

DIET NUMBER	CASEIN	SALTS	BUTTERFAT	LARD	YEAST	DEXTRIN
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	25	3.7	5	15	5	46.3
2	30	3.7	5	19	5	37.3
3	40	3.7	5	19	5	27.3

TABLE 3A

Reproduction records on diets of table 3

DIET NUMBER	NUMBER OF MALES	NUMBER OF FEMALES	NUMBER OF LITTERS	TOTAL YOUNG	YOUNG REARED	YOUNG DIED	YOUNG CONSUMED	CURVE OF GROWTH
1	3	7	1	5	1	4	0	++
2	3	9	0	0	0	0	0	++
3	2	4	1	4	0	4	0	++

often results. It may be due to an unknown chemical factor such as vitamin E, but it is our opinion that considerable more work must be done before such a conclusion is warranted. It is certainly true that we must take into consideration the difference in requirement of the individual

food components such as amino acids, inorganic ions and vitamins for growth as contrasted with the amount necessary for reproduction and rearing of young, and until those requirements have been established it is unsafe to ascribe the results to some unknown principle.

All of the animals came from our breeding colony. They consist of what appears to be a healthy group. There is practically no mortality of either adults or young on this ration. It is possibly true that there is a difference of vitality of the individuals, but nevertheless all of the females and males appear to grow normally and reproduce repeatedly on our growing ration and such difference of vitality is not revealed on this complete

TABLE 4

Composition of diets

DIET NUMBER	CASEIN	SALTS	BUTTERFAT	YEAST	DEXTRIN
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	15	3.7	5	5	71.3
2	20	3.7	5	5	66.3
3	22	3.7	5	5	64.3
4	25	3.7	5	5	61.3
5	30	3.7	5	5	56.3
6	40	3.7	5	5	46.3

TABLE 4A

Reproduction records on diets given in table 4

DIET NUMBER	NUMBER OF MALES	NUMBER OF FEMALES	NUMBER OF LITTERS	TOTAL YOUNG	YOUNG REARED	YOUNG DIED	YOUNG CONSUMED	CURVE OF GROWTH
1	2	8	7	47	35	12	0	+
2	2	3	5	25	9	16	0	++
3	2	4	3	13	8	5	0	++
4	2	3	3	16	0	16	0	++
5	3	4	5	34	4	30	0	++
6	2	7	5	30	8	22	0	++

ration. The casein used in these experiments was prepared by washing with water acidified with acetic acid. Dextrin was made by autoclaving starch moistened with 1 per cent of citric acid at 15 pounds pressure for three hours. The salt was that employed by McCollum (1914). The rats were placed on the experiments when they weighed from 50 to 60 grams. The composition of the diets is given in tables number one to eight. In table 1 the diets contain different levels of yeast, while in table 2 the yeast is constant, but lard has been included in the ration. In table 3 the amount of casein has been increased and lard is also present in these diets. The object of the diets in table 4 is to ascertain the influence of protein on reproduction and rearing of young. The diets in table 5 con-

tained Harris' yeast tested for its vitamin content. The diets given in all of the other tables contain Fleischmann's yeast. The diets in table 6 were fed to animals on screens. Diets in table 7 contain undried Fleisch-

TABLE 5

Diets containing Harris yeast

DIET NUMBER	CASEIN	SALTS	BUTTERFAT	YEAST	DEXTRIN
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	18	3.7	5	2	71.3
2	18	3.7	5	3	70.3
3	18	3.7	5	5	68.3
4	18	3.7	5	8	65.3
5	18	3.7	5	12	61.3

TABLE 5A

Reproduction records on diets containing Harris yeast in table 5

DIET NUMBER	NUMBER OF MALES	NUMBER OF FEMALES	NUMBER OF LITTERS	TOTAL YOUNG	YOUNG REARED	YOUNG DIED	YOUNG CONSUMED	YOUNG DIED WITH MOTHER	CURVE OF GROWTH
1	2	3	0	0	0	0	0	0	−
2	4	5	4	20	7	0	13	0	++
3	12	25	11	67	30	20	17	0	+++
4	12	24	23	161	100	22	30	9	+++
5	10	15	15	83	49	18	16	0	+++

TABLE 6

Composition of diets

DIET NUMBER	CASEIN	SALTS	BUTTERFAT	YEAST	DEXTRIN
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	18	3.7	5	3.5	69.8
2	18	3.7	5	5.0	68.3
3	18	3.7	5	2.0	71.3

TABLE 6A

Reproduction records on diets given in table 6

DIET NUMBER	NUMBER OF MALES	NUMBER OF FEMALES	NUMBER OF LITTERS	TOTAL YOUNG	YOUNG REARED	YOUNG DIED	YOUNG CONSUMED	CURVE OF GROWTH
1	2	7	0	0	0	0	0	+−
2	1	5	2	19	6	6	7	++
3	1	3	0	0	0	0	0	−

mann's yeast. The diets of table 8 were made so as to test the influence of varying quantities of the fat soluble vitamins on reproduction. The reproduction records and curves of growth are given in tables 1A, 2A, 3A,

etc. The results under 1A are for the diets given in table 1, the results in 2A are for the diets in table 2, 3A for those in table 3, etc. The data are in the main self-explanatory. In table 1A it is shown that a large number of

TABLE 7
Composition of diets

DIET NUMBER	CASEIN	SALTS	BUTTERFAT	YEAST	DEXTRIN
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	18	3.7	5	4	69.3
2	18	3.7	5	5	68.3
3	18	3.7	5	6	67.3
4	18	3.7	5	8	65.3

TABLE 7A
Reproduction records of diets in table 7

DIET NUMBER	NUMBER OF MALES	NUMBER OF FEMALES	NUMBER OF LITTERS	TOTAL YOUNG	YOUNG REARED	YOUNG DIED	YOUNG CONSUMED	CURVE OF GROWTH
1	1	3	1	6	0	0	0	++
2	3	7	5	24	1	23	0	+++
3	1	6	2	10	4	6	0	++
4	1	3	1	6	0	6	0	++

TABLE 8
Composition of diets

DIET NUMBER	CASEIN	SALTS	BUTTERFAT	YEAST	DEXTRIN
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	18	3.7	9	5	64.3
2	18	3.7	12	5	61.3
3	18	3.7	24	5	49.3

TABLE 8A
Reproduction records on diets in table 8

DIET NUMBER	NUMBER OF MALES	NUMBER OF FEMALES	NUMBER OF LITTERS	TOTAL YOUNG	YOUNG REARED	YOUNG DIED	YOUNG CONSUMED	YOUNG DIED WITH MOTHER	YOUNG DIED FOR LACK OF CARE BY MOTHER	CURVE OF GROWTH
1	2	6	5	27	22	5	0	0	0	++
2	2	5	4	18	12	6	0	0	0	+
3	2	7	13	80	40	27	0	7	6	++

young are born on synthetic diets containing Fleischmann's yeast as the only source of vitamin B. Rats on 5 per cent of this yeast have been carried through the sixth generation. The animals on 3.5 and 8 per cent of the

same yeast have been carried through the third generation. It is also evident from the results that a large proportion of the young die on these synthetic diets. Death almost invariably occurs during the suckling period indicating one of two or both possibilities, namely, a decreased flow of milk or a change in the chemical composition of milk. The diets employed by Evans and associates contained lard as one of the ingredients, and upon these diets reproduction was not normal; few if any young were produced. Table 2A gives the results obtained on diets recorded in table 2. All of these diets had lard as a component. The results show that reproduction is not normal on these diets, which is in harmony with the data of Evans and associates. The fact that positive results were obtained on diets in table 1 as revealed in table 1A shows that the inclusion of considerable amounts of lard in synthetic diets is not conducive to reproduction, although the animals may grow at the normal rate to full adult size. In tables 3 and 3A are given results on the diets containing lard and additional protein. The data are not appreciably different from those in table 2A. Additional protein did not modify the influence of the added lard. These rats were on the lard and high casein rations 6, 7 and 8 months respectively, and since they were 5 to 6 weeks of age when placed on the experiments ample time was allowed for accumulation of complete and satisfactory data. Only one litter of 8 young was born on the diets in table 2 containing lard and butterfat as the sole source of vitamin A. This litter was born on the diet containing 19 per cent of lard and 5 per cent of butterfat. This is the only litter obtained on the high lard diets in table 2. The mother of these young was placed on the experiment when 75 grams in weight, which is a much older rat than normally employed. Young were born when the mother had been on the experimental diet 7 weeks. This mother never had any more young. Table 4A gives the results obtained on diets with varying amounts of casein and 5 per cent of Fleischmann's yeast as the sole source of vitamin B. It was believed that more normal reproduction and rearing of young might result by the inclusion of more amino acids in the diet, since it is conceivable that the comparatively few litters obtained on synthetic diets and the high mortality might be due to a difference in requirement of amino acids and although casein at 18 per cent furnishes a sufficient quantity of these amino acids for growth still at this level it may be deficient for the added functions of reproduction and rearing of young. A large number of young were born on these diets, but the mortality of the young was high even though the casein content of these diets was as high as 40 per cent. Animals were carried through the third generation on diets containing 15, 20 and 30 per cent of casein and 5 per cent of yeast as the sole source of vitamin B. In table 5A are recorded the results obtained with Harris' yeast. This is the yeast employed by Evans and associates, and it is conceivable that the positive results obtained by us might be due

to the kind of yeast employed. Favorable results were obtained on reproduction by the use of Harris' yeast, and at the present time we have third generation young on 5, 8 and 12 per cent of this yeast in the diet. These rations have the following composition: casein 18 per cent, salt mixture 185, 3.7 per cent, filtered butterfat 5 per cent, Harris' yeast 5, 8 or 12 per cent, and dextrin to 100 per cent. It is a fact that on Harris' as well as Fleischmann's yeast the vast majority of the young die before they are weaned. In tables 6 and 6A are given results on screens. The animals were placed on screens so that they could not consume the feces. The data are by no means complete but do show that young are produced under these conditions. It has been shown in this laboratory by Nelson, Fulmer and Cessna (14) that yeast has the capacity to synthesize vitamin B and it is conceivable that such is the case with certain of the bacteria of the intestinal tract, not only as concerns vitamin B but also with relation to vitamin E, if such exists. Although young were born on 5 per cent of Fleischmann's yeast when the animals were placed on screens time has not permitted continuation of them beyond the first generation. We shall report on this phase of the subject later. Steenbock and associates (15) have shown that if rats be prevented from consuming their feces the amount of vitamin necessary in the ration for normal growth is considerably greater and these investigators believe that the vitamin content of foods has been estimated too high. This seems to be verified by the above figures. Tables 7 and 7A give the diets and results when undried Fleischmann's yeast was incorporated in the diets. Young were born and reared but the mortality of the young was high. Since reproduction and rearing of young is not normal on synthetic diets, it was thought that the yeast might undergo some change during drying to account for the failure observed. It appears that undried yeast is more potent than the dried. Table 8A gives the results obtained by increasing the fat soluble vitamin content of the diet. The results of these experiments are for the diets found in table 8. Increasing the amount of butterfat did not by any means entirely overcome the detrimental effects on infant mortality. Animals were carried through the third generation on the diets (table 8) containing 12 and 24 per cent of butterfat. It is a striking fact that reproduction is not possible on high lard diets, whereas it is possible on high fat diets containing butterfat instead of lard. This suggests a possibility of a relation between vitamins A or D or both and fat metabolism. It is possible equally good results may be obtained on high fat diets containing lard if the fat soluble vitamins are increased in the diet.

In this connection we desire to call attention to another interpretation of these experiments. Two groups of rats which are not recorded in the tables were given the following rations which for convenience are designated rations A and B. Diet A had the following composition: dextrin

52.3, casein 15, lard 19, butterfat 5, Fleischmann's yeast 5, and salt mixture 185, 3.7 per cent. Four females and 1 male were placed on this diet and were continued on the experiment from April 8 to December 22, 1924. Only one female had young and the litter of 3 was eaten shortly after birth. On November 20 four of the group of 5 showed xerophthalmia. On November 21 the ration was modified by replacing 14 of lard with the same quantity of dextrin. The eyes of 2 rats cleared up entirely. On December 5 all of the lard was completely replaced by dextrin. At this time 1 had died and 2 were on the verge of death. The fourth recovered completely after this change.

Diet B consisted of casein 18, dextrin 52, lard 15, Fleischmann's yeast 5, salt mixture 185, 5, and filtered butterfat 5. Three females and 1 male were continued on this ration from April 8 to November 15, 1924. All of the animals showed xerophthalmia September 4. The lard was replaced by an equivalent amount of dextrin September 9. All eyes cleared up. Two of the animals died September 15; the other two survived. These experiments show either that high lard diets owing to their high calorific value decrease the amount of food consumed to such a low level that 5 per cent of butterfat is not sufficient to ward off the symptoms of xerophthalmia or that lard in some way causes a destruction of this fat-soluble vitamin. Due to the fact that a very small amount of butterfat will prevent the appearance of the symptoms of xerophthalmia we inclined to the latter view. Previous to this work we have shown that high lard diets were not conducive to reproduction. Since the completion of this work Fridericia (1924–25) has shown that certain fats including lard cause a destruction of vitamin A which appears to be in harmony with our work. These facts must be taken into consideration by workers who claim a new vitamin for reproduction when based on data obtained on high lard diets.

There are many more conclusions to be drawn from these results, but they are so readily seen by studying the tables that the writers feel it unnecessary to devote more space in order to point them out. Certain facts deserve emphasis, however. Rats reproduce with Fleischmann's and Harris' yeast. Reproduction is not normal, however. Although a large number of young have been obtained as shown by the figures, and although some have been continued through the sixth generation, most of the females on the synthetic diets containing yeast as the sole source of vitamin B have only one litter and then apparently cease to have more young. This is by no means always true. Twenty females have produced two litters of young, eight females three litters and two, four litters. The experiments recorded were continued from 6 to 13 months in order to obtain complete reproduction records, so that the animals of the first generation were from $7\frac{1}{2}$ to $14\frac{1}{2}$ months old at the conclusion of the experiments. The number of males and females given in the tables include all of the animals employed in these

experiments. Subsequent generations after the first were not observed for reproduction records because as soon as a litter was born and reared by a female the young were continued and the preceding generation killed. The number of young recorded therefore would have been much larger. Results are entirely different on a mixture of naturally occurring food materials such as constitutes our growing ration. The females give rise to litter after litter without any apparent cessation until the end of their natural period of fertility. The number of litters produced on the synthetic diets is not only limited in most cases, but the vast majority of the young die before they are weaned. This is not because the young are weak or of low vitality. The young are perfectly healthy when born, which is proven by the fact that if the young produced on the synthetic rations be given to lactating females on the growing ration the young are weaned and grow normally to full adult size. On the other hand if the young from the females on the normal growing ration are given to lactating mothers on the synthetic diets they die during the suckling period. This indicates that milk secretion is not normal either quantitatively or qualitatively or both. It appears from the data so far accumulated that a larger number of young are weaned in succeeding generations on synthetic diets although the number to the litter is smaller.

Of interest in this connection is the influence of a single natural food upon reproduction and weaning of the young of the rat. Successful results on growth and reproduction have been obtained by the use of a single cereal grain as the only source of protein and vitamin B. Oats, wheat or rye when included in the diet to the extent of 93 per cent plus 1 per cent of NaCl, 1 per cent of $CaCO_3$ and 5 per cent of filtered butterfat give apparently normal reproduction and the number of young weaned closely approximates the normal. Further work will have to be done to answer the question why these single natural foods give such favorable results, whereas synthetic diets do not. These experiments answer one point which is of considerable importance in this connection, namely, that 5 per cent of filtered butterfat supplies a sufficient amount of vitamins A and D for normal growth and reproduction. Whether it is possible to decrease the amount of butterfat below this value we do not know. The unfavorable results obtained on the synthetic diets employed by us is therefore not due to a deficiency of fat-soluble vitamins. Emphasis of this is desirable because others besides ourselves have thought that the abnormal results on reproduction and rearing of young were due to inadequate supplies of the fat-soluble vitamins.

The results on diet 9 are of interest. On 12 per cent of yeast 61 young were born and 52 reared or a mortality of only 15 per cent. One female had two litters and the other two females each had three litters of young. The mortality on 16 per cent of yeast was higher but the results on 12 and 16 per cent of yeast are not comparable since the animals were not on the

diets the same length of time. It is to be noted that the mortality in the case of these two diets was composed of young consumed by the mothers and that those young escaping this violent death were all reared except 1 on the 16 per cent level of yeast. This taken in conjunction with the fact that more litters appear to be born on high yeast diets seems to indicate that the amount of vitamin B required for normal reproduction and rearing of young is very much higher than for growth, and the failure of other investigators to obtain normal reproduction may, in part, be due to this factor. We are now attempting to determine the amount required for normal reproduction and normal lactation.

The writers desire to thank the Fleischmann Company for supplying the yeast used in these experiments.

SUMMARY

1. Reproduction and rearing of young are possible on diets containing the two kinds of yeast employed in these experiments as the sole source of vitamin B.

2. The mortality of the young before weaning on these diets is high although rats have been carried so far through the sixth generation on Fleischmann's yeast and through the third generation on Harris' yeast.

3. The number of litters born on the yeast diets employed is limited whereas on mixtures of naturally occurring food materials rats are very prolific.

4. Rats can tolerate a high fat diet when butter fat is employed, but they do not reproduce on high lard diets even though these latter diets contain a sufficiency of vitamin A for normal growth and normal reproduction in the form of butter fat.

5. Xerophthalmia has occurred in rats receiving lard diets containing 5 per cent of butterfat as the only source of fat-soluble vitamins.

6. The failure of these diets for reproduction and rearing of young is not due to a deficiency of fat-soluble vitamins but may be due to an insufficiency of vitamin B and an inadequate quantitative relation between the known constituents of the diet.

7. It appears to the writers that data are by no means conclusive for the existence of unknown dietary factors for reproduction and milk secretion.

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DIET IN RELATION TO REPRODUCTION AND REARING OF YOUNG. II

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Received for publication January 9, 1926

Considerable data have accumulated in the last few years on the rôle of diet in reproduction and rearing of young. The problem is important and merits careful consideration because of an apparent correlation between the results obtained on animal experiments and infant mortality and failure of milk secretion in the human and domestic animals.

Evans and associates (1925) of the University of California have been ardent advocates of the idea that an unknown dietary factor known as vitamin E is necessary for reproduction. They say that rats grow well and appear healthy on synthetic food mixtures consisting of purified protein, salt mixture, fat, carbohydrate, fat soluble vitamins and vitamin B, but sooner or later sterility results. They have shown, moreover, that the sterility disease affects males and females differently. They have studied the male sterility produced by their diets very thoroughly and have observed anatomically and histologically the sequence of events that leads to this condition. They have been able to recognize broadly four stages in the development of male sterility. They state furthermore that male sterility responds sooner or later to serious changes in the seminiferous epithelium and in advanced cases to a complete destruction of this tissue. These investigators maintain that male sterility is difficult to cure although they say "proper prophylatic experiments are invariably successful." Evans and co-workers (1925) have shown that vitamin E deficiency causes a specific disturbance in the reproductive mechanism of the female. Oestrus, ovulation, fertilization and implantation are normal, but a deranged condition manifests itself with the death and resorption of the developing young.

In the preceding article on this general subject in this number of the JOURNAL it has been shown that rats reproduce on synthetic diets containing yeast as the only source of vitamin B. Animals were carried through the sixth generation on Fleischmann's yeast as a source of vitamin B and through the third generation on Harris yeast, the source of vitamin B employed by Evans and associates. It was shown in this work that although young are born reproduction is not normal, because the fe-

males on these rations are not nearly as prolific as those on mixtures of naturally occurring food materials such as constitute the growing and breeding ration employed successfully in this laboratory for the past six years. Not only are the females less prolific on synthetic rations, but there is a high mortality of the young due in all probability to a derangement in the physiology of milk secretion.

Evans and associates have been supported in their ideas by several investigators especially Sure (1923–24) and Mattill and Stone (1923). On the other hand data from this laboratory indicate that considerable more work must be done before it can be accepted that such a substance as vitamin E is the cause of failure of abnormal reproduction. Reference in this connection is also made to the work of Hoffman (1923) who obtained reproduction into the second generation on synthetic diets and the recent investigations of Hogan and Harshaw (1924) indicate that the question as to the existence of vitamin E still remains to be proven. Evans and associates have done a large amount of careful work on gross anatomical and histological changes involved in abnormal reproduction brought about through absence of vitamin E. They claim these changes are specific for the vitamin in question. We are unable to correlate this with data presented in the previous paper in which it was shown that large numbers of young were born on synthetic diets and some carried through the sixth generation. The fact that young are not reared on certain synthetic rations is considered by some investigators as sufficient evidence that another unknown factor is required for normal milk secretion. Sugiura and Benedict (1919) for instance, showed that certain diets were insufficient for normal lactation, although growth in the first generation was at the normal rate. Ten cubic centimeters of whole milk altered the diet so as to induce normal milk secretion, which fact led these investigators to believe that milk contains a new accessory food factor which is needed by the mother in order to produce an abundant flow of milk. It is possible as pointed out in the preceding article that there is such a substance as vitamin E and another unknown principle which acts as a galactagogue and is necessary for normal milk secretion, but we do not believe that there is sufficient evidence for these conclusions. One thing is certain, that reproduction and rearing of young are not normal on synthetic diets.

Since synthetic diets do not give normal results on reproduction, rearing of young and lactation, it seemed to the writers to be worth while to ascertain the influence of individual naturally occurring foodstuffs on these processes. It should be possible by this method to obtain a more clear idea of the rôle of individual dietary components and also to ascertain the relative value of individual foods for these purposes, and in so doing determine accurately the degree of safety of diets for normal functioning of the

above mentioned physiological processes. If a wide variety of different individual foods give identical results strong evidence will have been presented to show that the abnormalities as concerns reproduction and rearing of young are not due to a lack of some unknown factor but in all probability to a failure from the supplementary or quantitative standpoint. Abnormal lactation is a common complaint of the human today. To what extent this is bound up with diet is not known, but the similarity between this condition in the human and the results obtained through experiments on small laboratory animals is very striking. It is also true that before conclusions can be drawn as regards the existence of vitamin E or a rational explanation given of the abnormal processes referred to above the amount of the individual food factors must be determined for growth as contrasted with production of young and normal milk secretion.

The experiments recorded in this paper deal with the vitamin B content of seeds for growth, reproduction, and rearing of young. Cereal grains constitute the greater part of the diet of man and many animals and for this reason a study of these substances is of considerable practical as well as theoretical importance. All of the experiments were performed on rats. They weighed from 50 to 60 grams when placed on the various diets. The following seeds were used for these experiments: white and yellow corn, wheat, barley and rye. The diets had the following composition: casein 18 per cent, salt mixture 185 (1924) 3.7 per cent, filtered butterfat 5 per cent, the different seeds from 10 to 60.0 per cent (white corn was also used at a level of 73.3 per cent) and the remainder of the diet to 100 per cent was composed of dextrin. Casein was purified by washing with water acidified with acetic acid. No casein was employed which did not show the absence of vitamin B insofar as that is possible by the biological method. Dextrin was prepared by moistening corn starch with 1 per cent of citric acid solution and autoclaving the mixture at 15 pounds pressure for three hours.

The results of the experiments are given in the tables. The data are for the most part self-explanatory so that it is unnecessary to consume space in order to go into great detail. In many particulars the results are analogous to the data in the preceding paper, in which are recorded the experiments with yeast as a sole source of vitamin B. There is a greater tendency for more litters to be produced by each female on the rations employed in these experiments than on synthetic diets containing either Fleischmann or Harris yeast as the only source of vitamin B. Within 6 months on 10 per cent of wheat one female had 3 litters, one 2 litters, and another female had 1 litter. The fourth female on this diet died in pregnancy at the end of $4\frac{1}{2}$ months. On 15 per cent of wheat one female had 6 litters during the $10\frac{1}{2}$ months on the ration. One female on the 20 per cent wheat diet had 4 litters in 10 months, another 2 litters in 6 months,

when the latter mother died during parturition. On 25, 30, 35 and 40 per cent of wheat two females had 4 litters each, 3 females each had 3 litters, and 8 females each had 2 litters. On the rye diets one female had 6 litters, another 5 litters, 7 females each produced 4 litters, 12 females had 3 litters each, and 12 other females each had 2 litters. The results for the other seeds are very similar. The figures show that females on these diets are very prolific. The number of litters and young born to each female compares quite favorably to the number obtained on the growing and breeding ration employed by us. Due to the fact that the females were segregated when quite well along in pregnancy and not

TABLE 1

Reproduction records on wheat diets

PER CENT OF WHEAT	NUMBER OF FEMALES	NUMBER OF MALES	NUMBER OF FEMALES DIED	NUMBER OF MALES DIED	NUMBER OF LITTERS	TOTAL NUMBER OF YOUNG BORN	NUMBER OF YOUNG DIED	PER CENT MORTALITY	NUMBER OF YOUNG WEANED	CURVE OF GROWTH	MONTHS ON DIETS
First generation											
10	4	3	2	0	5	25	25	100.0	0	+	6
15	6	2	2	0	10	57	55	96.5	2	+	10
20	6	2	4	0	7	39	29	74.3	10	++	10
25	6	3	2	0	10	82	72	87.8	10	++	10
30	4	3	1	1	6	48	18	37.5	30	++	7
35	4	2	1	0	10	65	23	35.3	42	++	7
40	3	2	0	0	8	58	35	60.3	23	++	7
60	6	3	2	1	5	30	12	40.0	18	++	5½
Second generation											
20	4	3	0	0	5	37	37	100.0	0	+	3
30	4	3	1	0	9	60	28	46.6	32	+	5
35	4	4	0	0	7	45	3	6.6	42	+	6
40	3	2	1	0	6	44	27	61.1	17	+	4
60	4	2	1	0	6	39	9	20.5	30	+	5½

mated until the young were fairly well grown naturally reduced the number in many cases below that obtained from animals on the growing ration, since the latter are with males a longer period of time. These results appear to be in harmony with the idea of Evans and his associates, but to the writers it seems that more data are required before a rational explanation can be ascribed to the greater degree of fertility induced by these diets. There are many factors to be taken into consideration, and our experiments now in progress are calculated to analyze them.

The data show furthermore that a large number of young are born, and

that the mortality of the young is high on many of the diets. The mortality varies somewhat depending on the nature of the substance employed as the source of vitamin B. There appears to be no relation in many of these experiments between the mortality and vitamin B content. For instance, on yellow corn in the second generation the mortality is 79 per cent on 60 per cent of this seed and 58 per cent on the 35 per cent level. On the 40 per cent level of wheat the mortality was 60 per cent while on 30 per cent it was only 35 per cent. On the other hand, in the

TABLE 2

Reproduction records on rye diets

PER CENT OF RYE	NUMBER OF FEMALES	NUMBER OF MALES	NUMBER OF FEMALES DIED	NUMBER OF MALES DIED	NUMBER OF LITTERS	TOTAL YOUNG BORN	NUMBER OF YOUNG DIED	PER CENT MORTALITY	NUMBER OF YOUNG REARED	CURVE OF GROWTH	MONTHS ON DIETS
First generation											
10	4	3	0	0	10	70	70	100.0	0	+	7
15	5	3	1	0	9	53	53	100.0	0	+	6½
20*	5	2	2	0	11	56	53	94.6	10	++	6½
25	5	2	1	0	19	166	157	94.5	9	++	10
25†	4	2	0	0	9	71	63	88.7	8	++	5½
30	4	2	0	0	14	107	69	64.4	38	++	7½
35	4	2	0	0	15	95	70	73.6	20	++	7½
40	4	2	0	0	9	74	39	52.7	35	++	7
60	4	2	0	0	7	55	23	41.8	32	++	5
Second generation											
30	4	3	0	0	5	40	28	70.0	12	++	4½
35	5	2	0	0	8	56	40	71.4	16	++	4
40	4	3	0	0	7	43	18	41.8	25	++	4½
60	5	2	0	0	10	74	34	45.9	40	++	4½

* Twenty-eight young born on this diet together with 1 per cent of codliver oil. Seven young only were weaned.

† Contained 8 per cent butterfat.

case of rye the mortality in the first generation seems to vary inversely as the vitamin B content of the diet. The same is true in the case of barley. Of interest in this connection is the high mortality of the females on these different diets. Seventeen of the 58 females on the wheat experiments died with a mortality of 29.3 per cent while in the case of the other materials the per cent of deaths ranged as follows: barley 25 per cent, rye 7 per cent, yellow corn 26.7 per cent, and white corn 16 per cent. These females died during pregnancy or parturition. It seemed as though they had difficulty delivering the young. Many of the females would

produce a few young, but die during parturition without delivery of the remainder. They exhibited no evidence of pain. They were very quiet for some time before delivery and exhibited considerable inertia. This condition does not occur on the mixture of naturally occurring food materials which serves as our growing and breeding ration.

It is to be noted that on the diet containing 73.3 per cent of white corn as the only source of vitamin B the mortality of the young was 71.4 per cent. This is the highest amount of seed which can be included in the ration without varying the amount of casein, salts, or vitamins A or D in the form of filtered butterfat. We do not know if similar results will be obtained on diets containing the same quantity of the other products. These experiments are in progress, but of necessity it requires considerable time to gather such large amounts of data. This work will be published

TABLE 3

Reproduction records on barley

PER CENT OF BARLEY	NUMBER OF FEMALES	NUMBER OF MALES	NUMBER OF FEMALES DIED	NUMBER OF MALES DIED	NUMBER OF LITTERS	NUMBER OF YOUNG BORN	NUMBER OF YOUNG DIED	PER CENT MORTALITY	NUMBER OF YOUNG REARED	CURVE OF GROWTH	MONTHS ON DIETS
First generation											
10	4	3	0	0	14	87	87	100.0	0	+	7
15	5	2	1	0	14	74	74	100.0	0	+	7
20	5	2	2	0	10	58	58	100.0	0	++	7
25	5	2	2	0	16	78	73	93.5	5	++	7+
30	6	2	2	0	17	106	86	81.1	20	++	9
35	4	2	2	0	8	60	30	50.0	30	++	6
40	4	2	0	0	8	58	13	22.4	45	++	6
60	3	2	0	0	6	56	12	21.0	44	++	6

as soon as it is complete. The fact that on the 73.3 per cent level rearing of young is not normal may possibly be due to a maladjustment of the known dietary factors in the ration. It is not a question of a deficiency of fat-soluble vitamins for on oats, rye or wheat at a level of 93 per cent as the sole source of protein and vitamin B, plus 5 per cent of butterfat as the only source of fat soluble vitamins, together with 1 per cent of NaCl and 1 per cent of $CaCO_3$ animals reproduce and rear young normally and there is no mortality among the females. If the high mortality of the young on this level of corn is due to a deficiency of vitamin B then it appears very probable that the average human diet is very close to the borderline and in many cases far below it as far as the needs of this vitamin is concerned for normal milk secretion and rearing of young. Further work is necessary in order to explain the failure manifested by these re-

sults. Only in one case did reproduction and rearing of young approximate the normal and by normal is meant the performance manifested by our growing and breeding ration. In this case 43 out of 45 young were reared on a level of 35 per cent of wheat which was the source of vitamin B of the second generation animals. The mortality was much higher in the first generation on the same level. This may indicate a survival of the strongest individuals. There is no sure and simple method for the measurement of vitality and it is probable that certain diets might serve a useful purpose in this connection.

The data presented in this paper also show that young are produced

TABLE 4

Reproduction records on white corn

PER CENT OF WHITE CORN	NUMBER OF FEMALES	NUMBER OF MALES	NUMBER OF FEMALES DIED	NUMBER OF MALES DIED	NUMBER OF LITTERS	NUMBER OF YOUNG BORN	NUMBER OF YOUNG DIED	PER CENT MORTALITY	NUMBER OF YOUNG WEANED	CURVE OF GROWTH	MONTHS ON DIETS
First generation											
10	4	3	0	0	4	25	25	100.0	0	−	7
15	7	2	3	0	2	5	5	100.0	0	+	7½
20	6	2	3	0	7	28	28	100.0	0	++	7½
25	6	2	2	0	9	55	49	89.0	6	++	9
30	4	2	0	0	12	82	77	93.9	5	++	9
35	3	2	1	0	10	63	39	61.9	24	++	7½
40	4	3	0	0	11	72	56	77.7	16	++	10
60	4	2	0	0	12	84	71	84.5	13	++	7
50	4	2	0	0	9	55	38	69.0	17	++	8½
73.3	4	2	0	0	9	70	50	71.4	20	++	7
Second generation											
25	1	4	0	0	1	7	7	100.0	0	+	3½
35	5	2	0	0	6	37	16	43.2	21	++	5
60	4	2	0	0	7	42	29	69.0	13	+	7½

on diets containing an amount of vitamin B which is barely sufficient for normal growth and on quantities insufficient for this purpose. Ten per cent of wheat supplies an amount of vitamin B which barely suffices for normal growth and still on this amount of wheat one female had 3 litters, and another 2 litters. On 15 per cent of wheat, which is 40 to 50 per cent more than the quantity of vitamin B required for normal growth, one female had 6 litters in 10½ months, which is a very good record. On 10 per cent of yellow corn two females each produced 2 litters and the same occurred on 10 per cent of white corn. Ten per cent of white or yellow corn does not supply a sufficient quantity of vitamin B for normal growth.

It must be concluded that as far as reproduction is concerned the amount of vitamin B is no greater than that required for normal growth, although for normal reproduction, that is for the production of the normal number of litters and the normal number of young to the litter, 50 per cent more of vitamin B may be required. We have not determined if animals become sterile eventually on diets low in vitamin B. It is also apparent from these data that although reproduction occurs on the minimum amount of vitamin B required for normal growth, normal rearing of young is not possible on this amount of this vitamin. It is our opinion that this failure is associated with a derangement in the physiology of milk secretion.

TABLE 5

Reproduction records on yellow corn

PER CENT OF YELLOW CORN	NUMBER OF FEMALES	NUMBER OF MALES	NUMBER OF FEMALES DIED	NUMBER OF MALES DIED	NUMBER OF LITTERS	NUMBER OF YOUNG BORN	NUMBER OF YOUNG DIED	PER CENT MORTALITY	NUMBER OF YOUNG WEANED	CURVE OF GROWTH	MONTHS ON DIETS
First generation											
10	4	3	2	1	5	36	25	69.4	11	−	7
15	7	2	4	0	3	16	15	93.1	1	+	7
20	6	2	3	0	7	43	39	90.6	4	++	7
25	3	2	1	0	11	79	72	91.0	7	++	8½
30	4	2	0	0	12	67	40	59.7	27	++	9
35	4	3	1	1	8	58	45	77.5	13	++	9
40	4	3	1	0	11	69	63	91.3	6	++	10
60	4	2	0	0	8	54	28	51.8	26	++	6½
50	4	2	2	0	14	89	75	84.2	14	++	8½
Second generation											
20	1	2	0	0	1	5	5	100.0	0	+	3
25	3	3	0	0	3	18	18	100.0	0	+	4
30	5	2	0	0	7	41	29	70.7	12	+	4
35	2	4	1	0	3	17	10	58.8	7	+	5−
60	5	4	0	0	11	73	58	79.4	15	+	6

The amount of vitamin B required for normal milk secretion is therefore many times that necessary for normal growth or reproduction and is possibly even greater than the ratio of fat soluble vitamins for the same purposes. Of interest in this connection is the fact that some young were reared on the diets containing 10 per cent of yellow corn as the only source of vitamin B. This, as stated before, is an amount insufficient for normal growth. No young were weaned on 10 per cent of wheat, rye, barley, or white corn. Yet this amount is sufficient for normal growth in all of the latter named products except white corn. Not only are

young not weaned on the 10 per cent level, but 15 per cent of wheat, rye, barley and white corn is not conducive to rearing of young. Only 2 young were reared on this level and those on the wheat diet.

It is not impossible that for optimum nutrition of the animal body the several dietary factors must bear a certain relative concentration to each other. It is conceivable that reproduction and lactation may be more sensitive to variation from the optimum concentration so that, although growth may be at the normal rate, reproduction and rearing of young fall below the normal. Fulmer, Nelson and Sherwood (1921) have shown that for the optimum reproduction of the yeast cell individual inorganic ions must be present in a certain concentration. Any deviation from this optimum concentration leads to a decreased formation of yeast.

There is a difference in the vitamin B content of the seeds tested. White and yellow corn are not as rich in vitamin B as the other products. Normal growth was obtained with the two varieties of corn at the 15 per cent level whereas with wheat, barley and rye 10 per cent sufficed. Although the mortality of the young was high, animals have been carried through several generations on many of these diets. The third generation has been obtained on 40 per cent of wheat and 35 and 60 per cent of yellow corn. Animals have also been continued through the fourth generation on 35 per cent of wheat.

This work was supported in part by a grant from the Quaker Oats Company through Mr. Carl S. Miner of the Miner Laboratories, Chicago.

SUMMARY

1. Rats grow at the normal rate with wheat, rye, barley, white or yellow corn as the only source of vitamin B. White and yellow corn are not quite as rich in this vitamin as the other seeds.

2. Reproduction approximates the normal on these diets, but there is a high mortality of the young due in all probability to a failure in milk secretion.

3. A large number of females die during pregnancy or parturition on the diets of these experiments. The mortality varies with the nature of the seed used as the source of vitamin B, being highest in the case of wheat.

4. The amount of vitamin B necessary for normal lactation is much greater than that required for normal growth and reproduction. The amount of vitamin B required for reproduction is not much greater than that required for normal growth.

5. Rats are more prolific on these diets than on diets containing either Fleischmann or Harris yeast as the source of vitamin B.

6. The abnormal results are not due to a deficiency of fat soluble vita-

mins but may be due to a deficiency of vitamin B. If such is the case then it is very probable that the human is close to the borderline as regards the needs for vitamin B in relation to lactation.

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X-RAY OBSERVATIONS OF HUNGER CONTRACTIONS IN MAN

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Received for publication January 9, 1926

In 1915, Dr. L. L. Hardt and one of the present writers (R) made observations of hunger contractions in man and in dogs, as these were made visible to x-ray examination by the use of a bismuth-coated rubber balloon introduced into the fasting stomach. Recently through the courtesy of the x-ray department of the Baylor Hospital, the opportunity was offered of repeating this study. By the use of refinements of technique not available in the original study, we have been able to reduce certain erroneous factors that were of necessity involved in the original work.

Although one of the present writers (R) was the human subject of the x-ray examination in that earlier work, he has never been satisfied that with the method therein employed, one was wholly justified in stating that the contractions being recorded or observed were not in part due to mechanical stimulation by the mass of the bismuth-coated balloon. In that work, to render the hunger contractions visible in an x-ray examination, we coated a rubber balloon with a paste of vaseline and bismuth and then enclosed this within a second one. Such a balloon has a weight of about 25 grams. The gastric contractions were then recorded graphically with simultaneous x-ray observation. Under these conditions the very vigorous contractions recorded graphically were seen to consist essentially of "hyper-peristalses." At the time these observations were made, the graphic tracing, not published in the original paper, showed that these contractions were of type II or III according to Carlson's classification. Radiographs of this hunger hyperperistalsis are given in the original paper. In the light of the previous studies of Professor Carlson (2) on the nature of hunger contractions this was unexpected, for his work seemed to indicate that hunger contractions were probably tonic contractions of the fundus of the stomach.

Within recent years, the introduction of double coated films used with intensifying screens and the Potter-Buchy diaphragm, have rendered the roentogenologic differentiation of gas shadows within the gastro-intestinal tract, relatively simple. It therefore occurred to us that if a thin rubber balloon were introduced into the stomach and distended with air, as is used in the graphic registration of hunger contractions, it should be visible

on the x-ray film. This has proved to be a practicable method. The outline of the air-filled balloon is not sufficiently distinct to give the detail desired in a fluoroscopic examination but in a series of rapidly made radiographs, the sequence of events can be clearly shown.

There were certain other considerations, that to one who had personally experienced and studied this gastric activity in hunger, required explanation. Carlson and his associates have clearly shown that hunger contractions are inhibited by the introduction of food, water or acids into the stomach. Yet it was not an infrequent occurrence in the work of Hardt and Rogers to find that hunger contractions may appear on the graphic record, and be subjectively felt by the individual, before the stomach has completely emptied itself. This observation would be explained if hunger contractions could be shown to be vigorous peristaltic contractions forcing the last remnants of food from the stomach. This therefore suggested that observations be made of the fasting stomach during the hypertonic period of hunger contractility and then introducing into the stomach a very small amount of barium sulphate in water, in order to determine whether or not the subsequent contractions, be they peristaltic or otherwise, would give the subjective feelings associated with hunger contractions. This of course introduces an introspective element in the work.

Successful execution of this work requires that the following conditions be fulfilled. The air-filled stomach must be visible in the radiographs and the subject of the experiment must be so accustomed to swallowing the balloon and to the necessary x-ray manipulations, that no nausea or disturbing mental influence will inhibit the hunger contractions.

Methods. One of the present writers (R) was the subject of the work. After eighteen hours' fasting and a three mile walk, the thin rubber balloon was swallowed and when arrangements had been made for making a graphic record of the hunger contractions, he then lay down on the x-ray table. Arrangements were made for making a series of radiographs of the stomach.

The films were given half-second exposures using an 8 inch parallel spark gap with 40 milliamperes flowing through a fine focus, universal coolidge tube, used in conjunction with a Potter-Bucky diaphragm. A filter of $\frac{1}{2}$ mm. of aluminium was placed beneath the tube, so that a large number of films might be made without danger to the skin of the subject, who lay comfortably in the supine position on the radiographic table.

When hunger contractions appeared a series of radiographs was made during and between the contractions (fig. 1). In one instance we succeeded in getting four films during the course of a single hunger contraction.

The examination of these films shows that in this individual the type I hunger contraction consists essentially of a circular contraction that

involves the lower half of the stomach. The greatest degree of contraction is exhibited by a band of muscle near the junction of the lower and middle thirds of the stomach, possibly the "transverse band" of older physiologists. During this contraction, the fundus of the stomach was slightly dilated as compared to its size in the intervals between hunger contractions. This seemed to be due to the compression of the lower half of the stomach which forced the air backward to the fundus. A few shallow peristaltic waves were seen at intervals during a preliminary

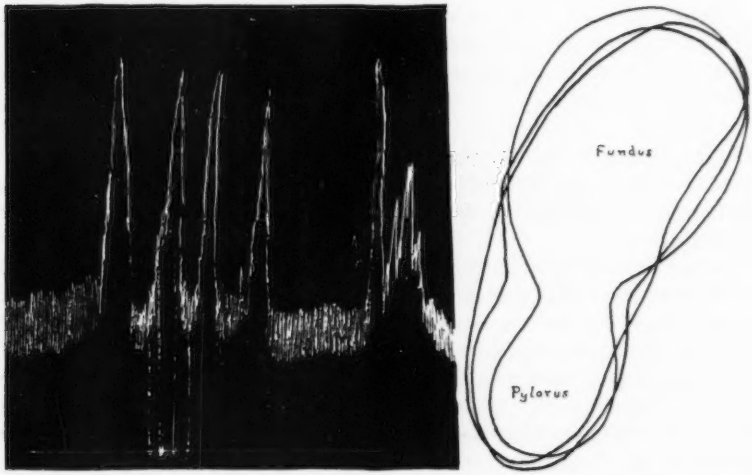

Fig. 1. Type I hunger contractions, after 18 hours' fasting, recorded by the rubber balloon method of Carlson. During the second contraction, four radiographs were made at the times indicated. The outlines of the stomach as observed roentgenologically, is shown to the right.

The three superimposed outlines indicate the contour of the stomach at the three instants of time marked *1*, *2*, *3* in figure 1. The fundus of the stomach is above, the pylorus is below. It will be observed that the contraction is a circular one of the lower half of the stomach and not a progressively advancing peristaltic wave. With a balloon in the empty stomach the long axis of the stomach is nearly transverse to the spinal column.

fluoroscopic examination, but these were certainly incidental as compared to the circular contractions. The appearance of the hunger contraction was similar to that sometimes spoken of by roentgenologists as a "local spasm" of the stomach.

We did not succeed in getting films of the period of tetany that sometimes terminates a period of hunger contractions.

This observation clearly showed that in this subject, a type I hunger

contraction as recorded by Carlson's method, consisted of a circular contraction in the lower half of the stomach.

We were then puzzled as to the hyperperistalsis that was observed in our previous work. It was suggested that possibly this hunger hyperperistalsis, in line with the work of Cole (3), was simply peristalsis in a hypertonic stomach due to the mechanical stimulation of the somewhat massive bismuth-coated balloons. It was therefore of interest to test this hypothesis by introducing into the stomach a small amount of barium sulphate, during a period of hunger contractility.

Three weeks later, after a twelve-hour fast, the subject again swallowed the rubber balloon and when hunger contractions were felt, he then swallowed 30 cc. of a mixture of barium sulphate and water. Radiographs of the stomach were made a few minutes later. In these films, it was found that the rubber balloon had been crowded into the upper part of the stomach and three very active peristaltic waves were playing over the lower two-fifths of the stomach. These peristaltic waves could be felt and were thought by the subject of the experiment to be weak hunger contractions.

Since the barium sulphate quickly sinks to the lower part of the stomach, we therefore repeated this experiment with a rubber balloon coated with a paste of vaseline and barium sulphate. This balloon had a weight of 27 grams. In this experiment type I hunger contractions were recorded and found to be associated with deep peristaltic waves running over the lower part of the stomach. Unfortunately in this particular experiment we were not able to see the fundus of the stomach and can not state whether or not, in this instance, there was any active participation of the fundus.

In this observation with the balloon coated with barium sulphate, vigorous peristaltic waves were seen running over the lower half of the stomach. The hunger contractions were vigorous ones and distinctly uncomfortable. In the observation with the air filled balloon the contractions were very definitely circular and not peristaltic in nature. It therefore seems that the subjective sensations associated with the hunger contraction may be induced by either type of muscular contraction if it be of sufficient intensity.

About an hour after this observation type III contractions appeared and these were radiographed. In this instance it was clearly seen that these contractions were peristalses. From this observation and the one recorded in our earlier report of work on this same individual, it seems that type III contractions consist of peristaltic waves superimposed on a hypertonic stomach. Type I contractions, as recorded by the rubber balloon method of Carlson, seem to consist essentially of circular contractions in the lower half of the stomach. If there be sufficient tonus in the stomach and sufficient mechanical stimulation by a heavy balloon, then vigorous

peristaltic waves also participate in the production of this type of hunger contraction.

From the introspective side, it has been observed that the subject stated he felt a hunger contraction when the x-ray films showed either very active peristalsis, or a circular contraction of the fasting stomach. The subjective feeling of hunger pain must therefore be conditioned on the intensity and prolongation of the contraction irrespective of whether it be circular or peristaltic in nature.

These observations have been made on a single subject only. It is therefore highly desirable to have them repeated on others. The resemblance of the circular contractions observed in the type I contraction to reflex spasm of the stomach known to occur in certain abdominal disorders, may be of clinical significance. This phase of the work we hope to present in a subsequent report.

The vigor of the gastric activity during hunger as observed by this method, was such that the roentgenologist suspected an abnormality of the stomach, although so far as known to the individual observed, there has never been any indications of gastric disorder. The routine clinical barium sulphate test meal (4 oz. barium sulphate in water sufficient to make 8 oz. of the mixture) as used in gastro-intestinal work was therefore swallowed and the stomach again examined. Under these conditions, from the clinical aspect, the stomach appeared to be normal. It may be of interest to call attention to the fact that with the balloon in the stomach, this organ is placed high up under the diaphragm with its long axis transverse to the long axis of the body, in a position quite different from that assumed to be normal when barium sulphate has been put in the stomach.

SUMMARY

If a thin rubber balloon is introduced into the stomach and slightly distended with air, as is used in the graphic registration of gastric contractility during hunger, there is sufficient differentiation of shadow density to make the outline of the fasting stomach visible on an x-ray film.

The type I hunger contractions, of Carlson's classification, were observed to be essentially strong circular contractions of the lower half of the stomach, involving particularly a band of muscle near the junction of the middle and lower thirds of the stomach. This type of hunger contraction may or may not be associated with active peristalsis. Type III contractions consist of active peristaltic waves running over a hypertonic stomach.

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RESPIRATORY CHANGES DURING AN AIRPLANE FLIGHT TO HIGH ALTITUDES

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Received for publication January 12, 1926

Occasionally doubt is expressed as to whether the effects of a flight to a high altitude are the same as those produced when these altitude changes are simulated by means of the low pressure chamber or by the rebreathing method of producing anoxemia. It will, therefore, be of interest to follow some of the changes of respiration during a flight and to compare them with those obtained by methods that are used to simulate altitude. Changes in the composition of the alveolar air of the lungs give information regarding the extent of ventilation of the lungs, the supply of oxygen, and chemical changes in the blood. Hence, we have determined the changes in the alveolar carbon dioxide and oxygen during flights to an altitude of 15,000 feet.

It is difficult to make satisfactory physiological observations during a flight in an airplane because of the cold and wind. For our study we used the Haldane-Priestley method of sampling the alveolar air. The samples were drawn into vacuum tubes. Considerable difficulty was experienced at first in finding a lubricant for the stopcocks that would withstand the cold. We finally found that the stopcock grease no. 47, put up by the Pyrolectric Instrument Company, fulfilled the needs fairly well.

The sampling tubes were evacuated with a Cenco-Nelson 3-stage pump; and the first, last, and one other of each series were tested for thoroughness of evacuation by filling with mercury. In several instances, some of the tubes were not used in the flight and were tested upon returning from 15,000 feet and found to be vacuous.

Most of the flights were made in the morning and always two or more hours after eating. Preliminary to the airplane taking off, several normal samples of alveolar air were obtained, the subject having been first thoroughly familiarized with the principle of alveolar air sampling. Ordinarily the ascent was made as rapidly as the airplane was capable of climbing to an altitude of 15,000 feet, which altitude having been attained was then held for 15 or 20 minutes. The alveolar air was sampled at regular intervals throughout the entire flight, and frequently even for several

intervals after landing. A number of gaps occur in our data because of loss of the seal of the vacuum tubes. The data were obtained from five subjects in fourteen flights.

The sensations of the subjects when at 15,000 feet were not alone those of anoxemia, although there were observed an increase in breathing, especially frequent deep sighs, and occasionally periodic breathing of the Cheyne-Stokes type. One subject developed a headache, another was nauseated and vomited several times, and still another noticed that slight exertion produced dizziness. All found the effects of the cold quite severe; the temperature in the cockpit frequently fell to between 10° and 15°F. The face, neck and hands were most affected; but the legs and feet were also often noticeably cold.

TABLE 1

The alveolar air tensions in mm. Hg during airplane flights

ALTITUDE	R. W. C.		H. H. F.		C. A. P.		L. M. T.		AVERAGE	
	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2
feet										
0	43.7	96.8	40.2	99.5	39.9	104.6	39.6	101.2	40.9	100.5
2,000			37.7	100.0	41.8	95.9	41.0	97.1	40.1	97.7
3,000	36.2	92.3			42.6	88.4			39.4	90.4
4,000			35.3	90.5					35.3	90.5
6,000	34.5	76.5	34.7	83.0	41.6	72.3	35.7	80.6	36.6	78.1
8,000	31.9	76.6	35.4	64.2	40.7	54.9	34.7	73.1	35.7	67.2
10,000	29.0	69.8	35.5	58.5	39.6	54.9	38.5	62.2	35.7	61.4
12,000	29.1	59.3	35.8	56.8					32.5	58.1
14,000	27.1	57.2	33.6	50.4			32.2	51.5	31.0	53.0
15,000	25.6	54.0	28.3	50.7	27.9	51.2	35.0	43.2	29.2	49.8

The average alveolar air tensions are given for four subjects in table 1. The averages of all our data, which have been arranged according to time and altitude, during the ascent and during the period of maintained altitude, are shown graphically in figure 1. The alveolar oxygen tension falls steadily during an ascent. In our experiments it required an average of 45 minutes to reach the altitude of 15,000 feet. During this interval the oxygen tension fell from an average of 100.5 mm. at sea-level to 49.8 mm., a decrease of 50 per cent, at the time the maximum altitude was reached. Following this, during a period of something over 15 minutes, while the altitude of 15,000 feet was maintained, the alveolar oxygen tension showed a tendency to increase; it rose to an average of 56.6 mm.

This fall in the oxygen tension in the lungs is sufficient to account for the symptoms of anoxemia experienced by our subjects during the flights. During the ascent the saturation of the blood with oxygen must have fallen from approximately 96 per cent to 82 per cent; after which, during the period of maintained altitude, it again rose to about 86 per cent.

A fall in the alveolar carbon dioxide tension also occurs during an ascent. While our data do not give a smooth curve, it is probably true that the tension falls gradually and smoothly as the ascent is made. It fell in our experiments from an average of 40.9 mm. at sea-level to 29.2 mm., a decrease of 28.6 per cent, when the altitude of 15,000 feet was first reached; and continued to fall until it reached an average of 25.4 mm. at the close of the 15 minutes of maintained altitude.

Since the fall in alveolar carbon dioxide tension is caused by the washing out of this gas from the blood, due to increased breathing, it is evident that the respiratory center in the brain was stimulated by a lack of oxygen early in the flight. The averages indicate that the breathing was already

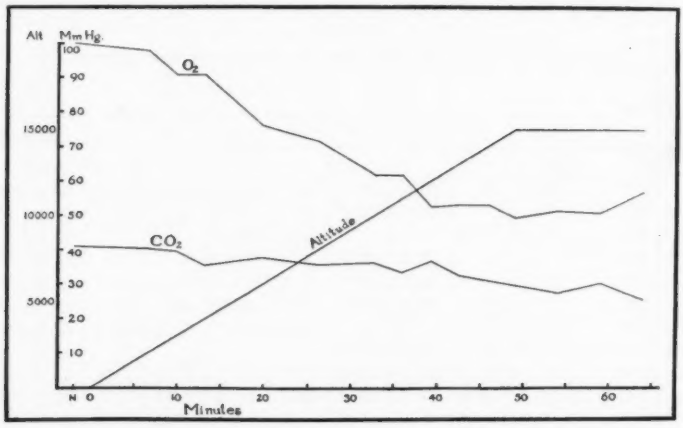

Fig. 1. Average alveolar air tensions of 5 men during 14 flights to an altitude of 15,000 feet.

slightly augmented at altitudes of 2,000 and 3,000 feet. This was clearly the case with two of our subjects, R.W. C. and H. H. F. In the case of H. H. F. at 2,000 feet, the carbon dioxide tension had already fallen from 40.2 mm. to 37.7 mm; and in that of R. W. C. at 3,000 feet, it had fallen from 43.7 mm. to 36.2 mm. However, that the respiration of all men is not equally responsive to changes in altitude is evidenced by the case of C. A. P. whose volume of breathing was not increased until 10,000 feet had been passed.

In a study of the after-effects of several flights, it was demonstrated that some time is required to make good the carbon dioxide lost while in the air. In two flights by H. H. F., the following changes occurred: *1*, before, 40.4 mm.; at 15,000 feet, 28.7 mm.; and 20 minutes after landing, 36.1 mm.; and *2*, before, 41.9 mm.; at 15,000 feet, 32.5 mm.; and 15 minutes

after landing, 36.4 mm. In two flights by L. M. T., the following were obtained: *1*, before, 38.7 mm.; at 15,000 feet, 30.6 mm.; and 15 minutes after landing 37 mm.; and *2*, before, 40.5 mm.; at 15,000 feet, 28.9 mm.; and 20 minutes after landing, 38.8 mm. In each of these cases the minute volume of breathing must therefore have been subnormal, not only during the 15 minutes observed but for sometime longer, in that the alveolar carbon dioxide tension was still below normal at the time of the last determination.

Our alveolar air data for flights show marked similarity when compared with the results obtained by Lutz and Schneider (1919) on men subjected to low oxygen tensions by means of a low pressure chamber and by rebreathing. Lutz and Schneider determined the average alveolar air tension for 24 cases at pressures comparable to altitudes of sea-level, 4,000, 8,000, 12,000, 16,000 and 20,000 feet. The oxygen tension at these pressures averaged 103.2, 83.7, 66.0, 53.3, 42.6, and 34.8 mm. respectively; the carbon dioxide tension averaged 39.7, 37.0, 36.2, 33.6, 31.3, and 30.0 mm. respectively. They also subjected some of their subjects to the same low oxygen tensions by the rebreathing method and obtained practically identical results. Our flight data for the oxygen changes give a curve similar to those obtained by Lutz and Schneider. These are so nearly alike that they might well pass as representations of the same phenomenon. There is, however, a slight difference that may have some significance. The alveolar oxygen tension during flights is always a trifle higher than when men are subjected to the same atmospheric oxygen tensions by the other methods. Thus during flight the average alveolar oxygen tension at altitudes of 4,000, 8,000, and 12,000 feet was 90.5, 67.2, and 58.1 mm. respectively; at comparable pressures in the low pressure chamber they were 83.7, 66.0, and 53.3 mm. respectively. From data obtained on Pikes Peak, altitude 14,110 feet by the Anglo-American Pikes Peak Expedition (Douglas, Haldane, Henderson and Schneider, 1913) and Schneider (1923) we find that the average alveolar oxygen tension of eight men during the first three to seven hours spent at that altitude was 45.8 mm. From the data of Lutz and Schneider on men in the low pressure chamber, we find the average at a barometric pressure comparable to an altitude of 14,000 feet was 48.0 mm. The average for the airplane flights at an altitude of 14,000 feet was 53.0 mm. This slightly more favorabe degree of alveolar oxygen pressure that is present during flights is probably the result of wind action. Aggazzotti and Galeotti (1919) found that wind favored lung ventilation and lowered the alveolar carbon dioxide tension.

The rise in alveolar oxygen tension that occurs when an altitude is maintained during a flight is similar to changes observed by Lutz and Schneider during the first part of a hold in the low pressure chamber. In both experiences the breathing for a short time continues to increase, during which

time more carbon dioxide is ventilated out of the blood, the alveolar carbon dioxide tension is further reduced, and the oxygen tension is raised.

A comparison of the carbon dioxide changes, due to the several methods of inducing anoxemia, shows the curves to be almost identical; but in conformity with the slight difference in oxygen there is a very slight difference in the carbon dioxide pressures: the pressures during flights were less than during exposure to the low oxygen of the low pressure chamber and of rebreathing. Thus Lutz and Schneider obtained on men in the low pressure chamber, at pressures comparable to altitudes of 4,000, 8,000 and 12,000 feet, alveolar carbon dioxide pressures of 37.0, 36.2 and 33.6 mm. respectively; while during flights at the same altitudes, they were 35.3, 35.7 and 32.5 mm. respectively. From the Pikes Peak (14,110 feet) data, we find that the alveolar carbon dioxide during the early hours averaged 32.6 mm.; for the same simulated altitude in the low pressure chamber data it averaged 32.5 mm.; and at the same altitude in airplane flight it averaged 31.0 mm.

The early response of the respiratory center to the increasing altitude of the airplane ascent corresponds to the rebreathing response reported by Schneider (1918), and Ellis (1919), and to the low pressure chamber response observed by Lutz and Schneider (1919). Schneider found that in a few men the lung ventilation begins to increase with the first decrease in the percentage of oxygen in the air breathed; while Ellis and Lutz and Schneider observed that a majority of all men increase their breathing by the time the reduction in oxygen pressure corresponds to an altitude of 4,000 feet.

The after-effect of a flight on the breathing is the same as that observed following other experiences of anoxemia. Schneider, Truesdell and Clarke (1925), from experiments conducted in a low pressure chamber, reported in the after period an immediate reduction in the minute-volume of breathing and in the output of carbon dioxide, so that for awhile these were subnormal. The time required for a return to normal ventilation depended upon the degree of anoxemia that had been experienced and the length of the exposure.

It appears, therefore, that the respiratory changes that occur during and after airplane flights to high altitudes, with the exception of a slightly favoring effect exerted by the wind produced by the propeller, are those of anoxemia; and that these can be reproduced by methods of simulating altitude.

SUMMARY

1. The alveolar air was sampled and analyzed for five men who took a total of 14 airplane flights to an altitude of 15,000 feet. In these cases the average alveolar oxygen tension fell 50.7 mm., 50 per cent; and the alveolar carbon dioxide 11.3 mm., 28 per cent.

2. The alveolar oxygen tension again rose an average of 6.8 mm. in a period of 15 minutes during which the altitude was maintained.

3. The ventilation of the lungs was increased in some instances at altitudes of 2,000 and 3,000 feet, but in one case not until 10,000 feet had been passed.

4. The alveolar carbon dioxide tension remained below normal for some time after the flight. It had not returned within 15 minutes after four flights.

5. A comparison of the respiratory conditions of airplane flights with those of anoxemia produced in other ways shows that the changes are chiefly those of lack of oxygen. In airplane flights the alveolar oxygen tension is slightly higher than under conditions of simulated altitude. The difference is attributed to the favoring action of wind on pulmonary ventilation.

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STUDIES ON THE PERMEABILITY OF LIVING CELLS

VI. The Penetration of Certain Oxidation-Reduction Indicators as Influenced by pH; Estimation of the rH of Valonia[1]

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Received for publication December 28, 1925

In a series of studies on the permeability of the living cell the penetration of acids, alkalis, bases and arsenic into the large unicellular marine alga, *Valonia*, has been tried. The experiments were begun at Miami, Florida, on *V. ventricosa* Agardh. (1), (2), (3), (4) and continued at Bermuda on another species, *V. macrophysa* Kütz (5). *V. ventricosa* occurs as single spherical cells, but *V. macrophysa* grows in clumps which can be easily separated into the individual cells. Owing to crowding, the latter are elliptical in shape. They grow attached usually to coral rock or some other firm foundation. *V. ventricosa* is much larger in size than *V. macrophysa* and grows on sandy weedy bottom.

Valonia is a one-celled marine plant with a delicate layer of protoplasm lying just inside the cell wall. The central vacuole is filled with sap which can be easily expressed and analyzed. In this way, one can make direct determinations of what passes through the protoplasm into the sap. When studying the penetration of arsenic (5), it was possible to recover from the wall, protoplasm and sap respectively the amount of arsenic penetrating. This is of course the most direct method of finding out what happens in the protoplasm. When data do not include analyses of protoplasm, but of sap only, the conclusions must of necessity be limited in their scope.

The present paper[2] deals with the penetration of some of the oxidation-reduction indicators into the sap of *Valonia*. The colorimetric method used did not permit measurements of the amount of dye in the protoplasm. The dyes were prepared and their physical-chemical characteristics studied by Clark et al, (6) (7), (8), (9), (10)whose data have been of great assistance in analyzing the experimental results.

The experiments were carried on directly in front of a window from which all the plants received substantially the same amount of diffuse illumination. The temperature, also, was a very important factor, as slight differ-

[1] Published by permission of the Surgeon General.

[2] Abstract published in Proc. Soc. Exper. Biol. and Med., xxiii, no. 4, Jan. 1926.

ences in temperature produced great differences in the amount of dye found inside the cell. For this reason, only those experiments which were done at the same temperature could be compared. The temperature in the laboratory throughout the season varied from 19° to 25°; however, no set of experiments done on the same day was ever subject to a temperature variation of more than a small fraction of 1 degree.

Both external and the internal H ion concentrations were varied in the experiments: the former by the use of buffers and the latter by placing the cells in sea water containing CO_2 or NH_3.

It has been previously demonstrated by the writer (2) that when *Valonia* is placed in sea water containing free CO_2, free CO_2 enters the sap of these plants producing a greater acidity than obtains under normal conditions. This method has been used in this paper to increase the acidity of the sap. Since this work was done Osterhout and Dorcas (11) have published a quantitative estimation of the penetration of CO_2 into *Valonia*.

Hoagland and Davis (12) have shown that by placing *Nitella* in solutions containing 0.005 M ammonium salt, the pH of the sap increases. The writer (3) has shown that when *Valonia* was placed in various concentrations of ammonium salts, the pH of the sap varied according to the concentration of the salt used, and furthermore proved that the increase in pH was due to the presence of ammonia as shown by the use of Nessler's reagent. Following these observations, McCutcheon and Lucke (13) showed that a less amount of brilliant cresyl blue penetrates into the sap of *Nitella* when ammonium salt is used in the external solution than when NaOH is present. Still later, Irwin (14) verified McCutcheon and Lucke's observations, using the writer's methods for varying the pH of the sap with various concentrations of ammonium salts in the external solution, and estimating the concentration of ammonia in the sap by Nessler's reagent as was suggested by the writer.

Thus for each pH value of the external solution three sets of plants were used: those which were normal; those which had been in CO_2 and those which had been in NH_3 solution.

When buffers are used it is advisable to check the results, lest differences be attributed to H ions when the buffer salts themselves are in reality responsible. At pH's 5.0 to 6.2 phthalate buffers (of Clark (15)) were found injurious to *Valonia*. At pH's 7.0 to 8.0 the use of phosphate buffers (of Clark) led to a greater accumulation of dye in the sap than did Palitzsch's borax buffers. In this connection it may be well to add that with borate buffers there is a disturbance of the oxidation-reduction equilibria of indigo disulphonate (Sullivan et al) (8). The well-known boric acid-mannitol complex is another example of an effect due to the buffer salts rather than the pH. These cases indicate the importance of fully considering the effect of the buffers themselves in all H ion studies.

Clark's citrate buffers appeared by comparison with other buffer mixtures to have no effects attributable to the buffer salts, and were therefore used for the 2,-6,-dibromo phenol indophenol and the indigo di- and tetrasulphonate experiments. For methylene blue, borax buffers also were used. Since the results so obtained did not differ from those obtained with citrate buffers in the pH range in which they overlapped, it is probable that the kind of buffer used in this case made no material difference in the results.

The concentration of the buffer in sea water was 0.0087 M. The pH of the sea water was adjusted by citrate buffers to pH values varying from 5.0 to 7.5; with borax buffers the pH ranged from 7.0 to 9.0.

To change the pH of the sap, from normal (6.4) to 5.2 the plants were placed for 20 minutes in sea water through which enough CO_2 from a tank had been bubbled to bring the pH of the sea water to about 6.0. This treatment did not injure the plant as was shown by subsequent survival experiments.[3]

To produce an alkaline reaction in the sap, the plants were placed for fifteen minutes in sea water to which enough dry NH_4Cl had been added to produce a concentration of 0.0025 M. This changed the pH of the sap from 6.4 to 9.0 without permanent injury to the plant as was shown by subsequent survival experiments.

No correction for salt error was made in any of these H ion determinations.

The initial pH values of the sap of those plants which had been in CO_2 solution or in NH_4Cl solution were not maintained throughout the experiment: after the first hour the pH gradually changed towards that of the untreated plants whose sap gradually became more acid when the external pH value was 6.2 or less. When the external pH was more than 6.2 there was no change in the pH of the sap of normal untreated plants during the time of experimentation.

The dyes used were: the Na salt of 2,-6,- dibromo phenol indophenol which has a m. w. of 379, and which in the case of the sample used contained about 10 per cent of moisture; methylene blue, m. w. 320, sample F, which contained 99.4 per cent dye on the basis of nitrogen content, 100 per cent on basis of moisture content and 98.2 per cent as calculated from titanium titration before drying; K-indigo tetra sulphonate with a m. w. of 702 and gave 95.01 per cent dye content when titrated with titanous chloride and K-indigo disulphonate with a m. w. of 498. The dyes were all prepared in the Division of Chemistry at the Hygienic Laboratory and were of special purity. It is of course most important to have highly

[3] Irwin (Journ. Gen. Physiol., 1925, viii, 147) states that she was not able to change the pH of the sap of *Nitella* by placing the plants in CO_2 dissolved in distilled water. It is very simple to demonstrate this both in *Nitella* and *Valonia* provided precautions are taken to prevent the escape of CO_2 before measuring the pH of the sap.

refined dyes, otherwise results erroneously attributed to the dye may be due to the impurities present.

The solutions in which the plants were immersed consisted of sea water to which had been added buffer solutions and dye. The dyes were dissolved in a small amount of distilled water and enough of this added to the sea water to make the concentration of dye in sea water 0.00035 M.

The three sets of plants with different internal pH values were placed in a series of solutions whose pH values varied from 5.0 to 7.5, and allowed to remain there ten hours. At the end of one, three, five and ten hours some were taken out of the solution, quickly rinsed to free them of all contaminating dye, wiped on filter paper, pierced by a sharp piece of glass and the sap expressed into a small glass tube. The sap is under pressure and comes out readily.

When indophenol dyes are used the dye appears in the freshly expressed sap in a reduced state. The dye may be oxidized by the addition of a trace of NaOH and slight shaking, and then assumes its original blue color. Shaking alone, or placing the tube containing the sap in sunlight will also oxidize the dye which it contains, but more slowly. Methylene blue was not found in the freshly expressed sap in the reduced form under the experimental conditions used. Not enough indigo tetra- or di-sulphonate penetrated to make accurate measurements possible.

The concentration of dye in the sap is determined by comparison of its color with a set of standard solutions of the dye. The lowest concentration of the indophenol which could be distinguished from distilled water was 0.000001 M.

It is of interest to note that the concentration of 2,-6-, dibromo phenol indophenol in the sap never equalled that of the external solution. In fact when a concentration of even 0.00005 M was found in the sap, it was always in the oxidized form and the cell did not survive.

It is also important to ascertain whether or not the plants had been injured by the treatment. Normal plants remain in good condition in the laboratory at least a month when the sea water in which they are kept is changed every day. It has been found by previous experience that those plants which were injured by treatment, cytolyzed within a month. Survival experiments of plants of *Valonia* treated with these dyes as indicated in the experiments showed that there was no injury except at pH 5.0. At this pH a small percentage of the plants did not survive a month, showing that there was some injury.

1. Results with 2,-6,- dibromo phenol indophenol: a. Analysis of the reaction velocity. The course of the process of penetration of 2,-6,- dibromo phenol indophenol into the sap of *Valonia* at different pH values of the solution surrounding the cells is shown in table 1 and figure 1. The abscissae represent time in hours and the ordinates M concentration of

dye in the sap. The crosses indicate observed values, while the smooth curves are graphs of the equation for a bimolecular reaction (16),

$$K = \frac{1}{t}\frac{x}{a(a-x)}$$

in which x = amount of dye in the sap at time t; a = amount of dye in the sap at equilibrium, and K = the velocity constant. At pH 5.0, $a = 11 \times 10^{-6}$; at 5.6, $a = 10 \times 10^{-6}$; at 6.2, $a = 4.5 \times 10^{-6}$. The concentration of dye in the sap at equilibrium was determined by extrapolation of the observed curves rather than by direct observation, as longer exposure of the cells at low pH values resulted in injury. The curves were plotted for 3 pH values only (i.e., 5.0, 5.6 and 6.2), since the observed penetration at higher pH was so small. The agreement in the velocity constants for pH's 5.6 and 6.2 is fairly satisfactory while that for pH 5.0 is not quite so good, although the constant is of the same order of magnitude. The agreement of the observed time-penetration curves with the graph of a bimolecular reaction is better than that with any other simple reaction or small group of consecutive reactions What disagreement there is may be due to the injury of some cells at the low pH value. Ten hours is as long as most cells can be kept at pH 5.0 without irreversible injury, and

TABLE 1

M concentration of 2,-6,- dibromo phenol indophenol in the sap of Valonia when the external concentration is 0.00035 M at various pH values and temperature, 20°C. Average of 10 experiments

pH 5.0; $a = 11 \times 10^{-6}$				pH 5.6; $a = 10 \times 10^{-6}$			
t	x	$K = \frac{1}{t}\frac{x}{a(a-x)}$	calc. x	t	x	$K = \frac{1}{t}\frac{x}{a(a-x)}$	calc. x
1	70×10^{-7}	0.159×10^{6}	63×10^{-7}	1	52×10^{-7}	0.108×10^{6}	50.4×10^{-7}
3	84×10^{-7}	0.098×10^{6}	88×10^{-7}	3	70×10^{-7}	0.12×10^{6}	75.0×10^{-7}
5	98×10^{-7}	0.14×10^{6}	96×10^{-7}	5	82×10^{-7}	0.09×10^{6}	85.0×10^{-7}
10	100×10^{-7}	0.091×10^{6}	102×10^{-7}	10	90×10^{-7}	0.09×10^{6}	91.0×10^{-7}
Average.....		0.122×10^{6}		Average...		0.102×10^{6}	

pH 6.2; $a = 4.5 \times 10^{-6}$				pH 7.0; $a = 8 \times 10^{-7}$		pH 7.5	
t	x	$K = \frac{1}{t}\frac{x}{a(a-x)}$	calc. x	t	x	t	x
1	11×10^{-7}	0.072×10^{6}	10.6×10^{-7}	1	0	1	0
3	23×10^{-7}	0.077×10^{6}	21.0×10^{-7}	3	0	3	0
5	26×10^{-7}	0.062×10^{6}	27.0×10^{-7}	5	0	5	0
10	33×10^{-7}	0.061×10^{6}	34.0×10^{-7}	10	7×10^{-7}	10	3×10^{-7}
Average.....		0.068×10^{6}					

even within ten hours, some cells usually did not survive as long subsequent to treatment as normal cells. This set of experiments was carried on at a temperature of $20 \pm < 1°C$.

The fact that the time-penetration curves in these experiments resemble the time curves of bimolecular reactions leads us to inquire whether there is any possible bimolecular reaction which could limit the rate of penetration of the dye. Since the indophenol is found to be reduced before entering the sap, (freshly expressed sap is incapable of itself reducing the dye) it might be conjectured that it is reduced by reaction with some reductant present in approximately equivalent concentration in the protoplasm, and that only after reduction can it enter the sap. Exact agreement with a bimolecular reaction curve would be expected only when the dye and reductant were present in exactly equivalent amounts at the beginning of the reaction, and were neither of them replenished. With this in mind the approximate agreement of the observed curves with those of a bimolecular reaction is very suggestive. The reductant in the protoplasm is very probably the SH- group of glutathione which Hopkins (16) found to be a constant constituent of cells with active metabolism, and which, when added to methylene blue in a reduced form, reduced methylene blue. Voegtlin et al (20) further found that all these indicators are reduced by reduced glutathione. The question of a kinetic interpretation of the time-penetration curves will be referred to again.

b. Relation between undissociated dye in the outside solution and in the sap. The curve in figure 2 shows the per cent of undissociated oxidized dye present at the indicated pH values; the dissociation constant of the oxidized dye is 2×10^{-6} (9). The circles indicate the proportionate amounts of dye penetrating at the different pHs. They are obtained by extrapolating to equilibrium the amounts of dye on the time curves for each pH value (using values for "a" from table 1), plotting these values as ordinates against pH as abscissae, determining the inflexion point on the curve so obtained and calling this "50" on a new scale of ordinates running from 0 to 100. The values of the observed points on this new scale were determined and plotted as ordinates in figure 2. It is evident that the curve coincides with the dissociation curve, showing that the amount of dye in the sap is proportional to the amount of undissociated dye of the oxidized form on the outside. The same shape of curve is obtained when ordinates represent amounts of dye in sap at any time, showing that the rate of penetration is proportional to the amount of dye at equilibrium. There is a concentration gradient between the M concentration of the dye in the external solution (which is 1×10^{-4}) and that of the dye in the sap (which is 1×10^{-7}). There is also an oxidation-reduction difference between the outside solution, which is completely oxidized, and the dye in the sap, which is completely reduced.

Let us offer an hypothesis to account for these differences between the outside and the inside solutions. We shall assume that the E_h or oxidation-reduction potential and the pH of the protoplasm are not materially changed by differences in pH of the outside solution. We shall also assume that the concentration of dye in the protoplasm at equilibrium is equal to that of the undissociated molecules of the oxidized dye in the external solution, and that only a very small percentage of this amount is reduced at the E_h and pH values existent in the protoplasm because the protoplasm is "poised" with respect to E_h in the same way that solutions are buffered with respect to pH. In other words, the cell tends to maintain a constant ratio between oxidant and reductant in the protoplasm, and hence a constant reducing potential which is such as to tend to reduce but a very small proportion of the dye used. If the absolute amount of reductant be very small, it might well happen that the dye, as it enters the protoplasm, very quickly uses up the reductant present and that this is only slowly restored in amount by the occurrence of some reaction in the protoplasm. This is in agreement with the known high velocity of oxidation-reduction effects on such dyes. As fresh reductant is produced, it would be used up by the dye until the proportion of dye reduced was that characteristic of the equilibrium reduction potential of the cell. This reduced dye will be passed on into the sap until an equilibrium with the characteristic concentration of reduced dye in the protoplasm is reached. Since reduced dye only can proceed into the sap (this is found by experimental evidence) the amount of reduced dye in the sap may be of a very much smaller order of magnitude than that of the oxidized dye in the external solution.

Furthermore, since the proportion of dye reduced is constant, the amount of reduced dye in the sap will be proportional to the oxidized dye in the protoplasm at various pH values, and if only undissociated dye can penetrate the protoplasm, its curve should coincide with the dissociation curve (as it does) because dissociation is a function of pH. In this way the protoplasm would be in equilibrium with the amount of reduced dye in the sap on the inside, and with the amount of oxidized dye in the external solution on the outside.

The statement that only reduced dye can enter the sap is supported by the fact that fresh sap added to dilute solutions of the dye fails to reduce the dye. This probably means that the reduction is carried out in the protoplasm. It seems therefore that the mechanism regulating the penetration of reduced dye into the sap may be explained by supposing it to be a function of the product of the amount of undissociated dye in the outside solution and the reduction potential of the protoplasm.

This hypothesis differs from that suggested above to explain the similarity of the time curves of penetration to bimolecular curves only in considering the limiting function to be the reaction restoring the concentration

of reductant to its normal value, rather than the reduction reaction itself which is presumably so fast as not to affect the curve.

c. Effects of changing the pH of the sap. Figure 3 shows the dissociation curve of the dye (curve *I*) as compared with the relative amounts of dye found in the sap when the internal pH is more acid than normal (curve *II*) and more alkaline (curve *III*). The abscissae represent pH values of the external solution and the ordinates relative amounts of dye found in the sap. These amounts were calculated in the same way that the curves in figure 2 were calculated. In finding the inflexion point in the case of NH_3, the curve had to be extrapolated as only the lower half was represented.

It can be readily seen from curve *II* that plants previously treated with CO_2 had greater amounts of dye in their sap than the dissociation curve would indicate at the various pH values, and from curve *III* that plants previously exposed to NH_4Cl had smaller amounts.

If the internal pH affects the rate of penetration of the dye into the sap, the following analysis of conditions should hold provided we assume that only undissociated dye penetrates into the cell from the external solution.

When the pH of the external solution is 5.6, about one-half of the dye is in an undissociated condition. If the pH of the sap were also 5.6, then the same concentration or some definite relative concentrations of dye should be found inside and outside (one-half of the dye in each case being dissociated and one-half undissociated[4]). If, however, the internal pH is greater (e.g., 9.0), and the external pH is still 5.6, the same relative concentration of undissociated dye should still be found in the sap together with a 99 times greater amount of dissociated dye, because the dye is about 99 per cent dissociated at that pH value and we are assuming that the equilibrium between outside and inside concentrations of dye is still dependent upon the concentrations of undissociated dye only. In other words, one ought to find more dye in the sap at greater internal pH values. This however is not the case, as there is actually less dye present when the sap is more alkaline.

When the sap is more acid, (e.g., pH = 5.2) the same relative concentration of undissociated dye should still be found in the sap together with a smaller relative concentration of dissociated dye, since the per cent of dissociated dye at that pH is less. In short, one should find less dye in the sap at pH 5.2. This again is not the case, as there is actually more dye present.

The above explanation assumes that the pH of the protoplasm changes with the pH of the sap. It is more reasonable to assume, however, that the protoplasm is highly buffered and that therefore the pH of the sap is not an exact indicator of the pH of the protoplasm, and this allows us to explain the apparently contradictory results in a wholly satisfactory way.

[4] The first dissociation constant of the dye is 2×10^{-6} (9).

These differences in the amount of dye penetrating may be explained by supposing that the cells were immediately surrounded by a thin layer containing CO_2 or NH_3 respectively which had diffused out of them, thereby changing the H ion concentration of the liquid in their immediate vicinity

TABLE 2

pH	I		II		III	
	Normal	Ratios	With CO_2	Ratios	With NH_4Cl	Ratios
			1 Hour			
5	7.0×10^{-6}	1.0	6.0×10^{-6}	1.0	1.2×10^{-6}	1
5.6	5.2×10^{-6}	0.74	3.1×10^{-6}	0.51	1.0×10^{-6}	0.83
6.2	1.1×10^{-6}	0.157	2.8×10^{-6}	0.47	0	—
7.0	0	—	1.3×10^{-6}	0.21	0	—
7.5	0	—	1.0×10^{-6}	0.16	0	—
			3 Hours			
5.0	8.4×10^{-6}	1.0	9.0×10^{-6}	1.0	2.5×10^{-6}	1
5.6	7.0×10^{-6}	0.83	6.0×10^{-6}	0.66	2.5×10^{-6}	1
6.2	2.3×10^{-6}	0.27	4.1×10^{-6}	0.45	0.2×10^{-6}	0.08
7.0	0	—	1.4×10^{-6}	0.15	0	—
7.5	0	—	1.3×10^{-6}	0.14	0	—
			5 Hours			
5.0	9.8×10^{-6}	1.0	9.0×10^{-6}	1.0	4.1×10^{-6}	1
5.6	8.2×10^{-6}	0.84	7.0×10^{-6}	0.78	2.5×10^{-6}	0.61
6.2	2.6×10^{-6}	0.26	5.1×10^{-6}	0.56	0.5×10^{-6}	0.01
7.0	0	—	1.7×10^{-6}	0.19	0	—
7.5	0	—	1.6×10^{-6}	0.17	0	—
			10 Hours			
5.0	10.0×10^{-6}	1.0	10.0×10^{-6}	1.0	6.0×10^{-6}	1
5.6	9.0×10^{-6}	0.9	8.2×10^{-6}	0.82	4.0×10^{-6}	0.66
6.2	4.8×10^{-6}	0.48	6.6×10^{-6}	0.66	1.0×10^{-6}	0.16
7.0	0.7×10^{-6}	0.07	2.0×10^{-6}	0.2	0.5×10^{-6}	0.08
7.5	0.3×10^{-6}	0.03	1.7×10^{-6}	0.17	0	—

Table 2 containing molecular concentrations of dye in the sap of *Valonia* at different pH values after 1, 3, 5 and 10 hours, and the ratios between the amount of dye at different pH values within each period of time. I, indicates cells not previously treated; II, cells previously treated with CO_2; III, cells previously treated with NH_4Cl.

and consequently changing also the amount of undissociated dye in that part of the external solution which is in contact with the cells. Therefore, if the rate of penetration depends upon the amount of undissociated dye in the external solution, a different amount of dye would penetrate

into the sap than would be expected at the H ion concentration of the rest of the external solution, which is what one observes. CO_2 should make the contiguous layer more acid, especially at higher pH values, thus repressing dissociation of the dye, and increasing its concentration in the sap. NH_4Cl should have the opposite effects, and they should be more pronounced at lower pH values. This is just what was observed.

In the light of this hypothesis, it is interesting to compare the results on penetration of dye with those on the penetration of arsenic into *Valonia* (5) when the same set of conditions had been imposed upon the cell, namely, changing the internal pH of the sap. In the case of arsenic, no correlation was found between the amount of arsenic in the sap and the dissociation of the arsenic in the external solution. It would therefore be expected that CO_2 and NH_4Cl would not affect the entrance of arsenic in the same way that they do that of the indophenol, and as a matter of fact they do not.

When arsenic acid was used, and the sap had been made alkaline with NH_4Cl, less arsenic was found in both the sap and the protoplasm of treated cells at all pH values of the external solution from 5 to 9. When arsenious acid was used instead, more arsenic was found in the treated than in the untreated cells. Exosmosis of NH_3 must have taken place in the case of both arsenic and arsenious acids, but yet the amount of arsenic in the sap was less in one case and greater in the other. One is forced to conclude that the explanation developed for 2,-6,- dibromo phenol indophenol is inapplicable to the case of arsenic. This substantiates the previously suggested fundamental difference in the nature of the penetration process in the two cases, i.e., that of arsenic and of 2-6 dibromo phenol indophenol.

McCutcheon and Lucke (13) changed the pH of the sap of *Nitella* by the use of NH_4OH and noted that less dye was found there than under normal conditions. These experiments are, however, not comparable with those of the writer, because there were so many differences in experimental conditions. McCutcheon and Lucke used a basic dye, while that of the writer, 2,-6,- dibromo phenol indophenol was an acid dye; the former was not reduced in the cell, while the latter was; the former accumulated, while the latter did not; the external solution of the former was alkaline, and kept alkaline by the use of NH_4OH, while that of the latter was acid.

It would be interesting to note the penetration of 2,-6,- dibromo phenol indophenol into the sap of *Valonia*, while the cells are still in NH_4OH or NaOH as McCutcheon and Lucke did with brilliant cresyl blue and *Nitella*. This would allow us to decide as to the possibility of explaining the results by the effects of exosmosis of NH_3 or CO_2 upon the dissociation of the dye in the external solution immediately surrounding the cell. This the writer hopes to do in the future.

Figures 4 and 5 respectively represent time curves showing the penetra-

Fig. 1. Curves showing the penetration of 0.00035 M 2,6,dibromo phenol indophenol into the sap of *Valonia* at 20°C. when the pH's of the external solution are 5.0, 5.6, 6.2, 7.0 and 7.5. The abscissae represent time in hours and the ordinates M concentration of dye in the sap. Crosses, x, show the observed values. Each point on curve is the average of 10 experiments. Smoothed curve is calculated according to the equation for biomolecular reactions.

Fig. 2. Curve showing the agreement between the per cent of undissociated molecules in the external oxidized solution of 2,-6,-dibromo phenol indophenol at various pH values (crosses) and the proportional amounts of reduced dye found in the sap at equilibrium when the external solution has various pH values (circles). The abscissae represent pH values of the external solution. Equilibrium conditions are extrapolated from time curves of figure 1. Temperature 20°C. + >0.5°. First dissociation constant of the dye is 2×10^{-6}.

Fig. 3. Curves showing the effects of CO_2 and NH_4Cl upon the penetration of 2,-6,-dibromo phenol indophenol into the sap of *Valonia*. Abscissae represent pH values of the external solution; ordinates, the proportionate amounts of dye in the sap. Concentration of external solution, 0.00035 M. Temperature, 20°C. + >0.5°. Average of 10 experiments.

Fig. 4. Time curves showing the penetration of 2,-6,-dibromo phenol indophenol into sap of cells previously exposed to CO_2 when the external solution has various pH values. The abscissae represent time in hours; the ordinates M concentrations of dye in the sap when multiplied by 10^{-7}. Temperature 20° + >0.5°.

Fig. 5. Curves showing the penetration of 2,-6,-dibromo phenol indophenol into the sap of cells previously exposed to NH_4Cl when the external solution has various pH values. The abscissae represent time in hours; the ordinates, M concentrations of dye in the sap when multiplied by 10^{-6}. Temperature, 20° + >0.5°.

Fig. 6. Curves showing the rate of penetration of 2,-6,-dibromo phenol indophenol into the sap of *Valonia* when the external concentration of dye varies from 0.00015 to 0.000063 M (curves 1 to 4). Abscissae represent time in hours, ordinates M concentration of dye in the sap when multiplied by 10^{-7}. Smoothed curve represents calculated, and crosses, observed values. No calculations were made for curve 4. pH of external solution, 5.4. Temperature, 24.5 + 0.5°C.

Fig. 7. Curves showing the characteristic potentials of four dyes at certain pH values and ratios of $\frac{S_r}{S_o}$. Abscissae are pH values, ordinates E_h values. Curves *A* and *B* represent 2,-6,-dibromo phenol indophenol when ratios of $\frac{S_r}{S_o}$ are $\frac{0.1}{99.9}$; and $\frac{99.9}{0.1}$ respectively. Curves *C*, *D* and *E* represent methylene blue, K-indigo tetrasulphonate and K-indigo disulphonate respectively when the ration of $\frac{[S_o]}{[S_r]}$ is $\frac{99.9}{0.1}$. Curves *A* and *B* represent probable bounds of pH values of protoplasm. E_h of the protoplasm estimated as somewhere along curve *A*. The two crosses at pH 6.4 indicate the limits for the E_h of the sap (0.12 and 0.15). Curves are drawn from Clark's curves for E'_o of these dyes.

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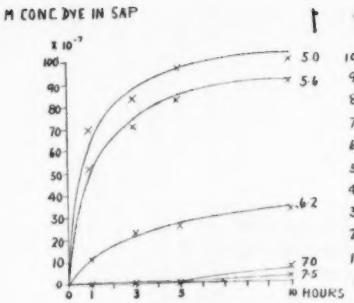

FIGURE 1

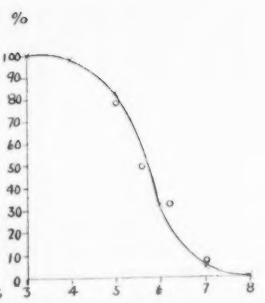

FIGURE 2.

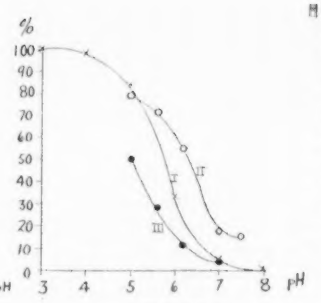

FIGURE 3

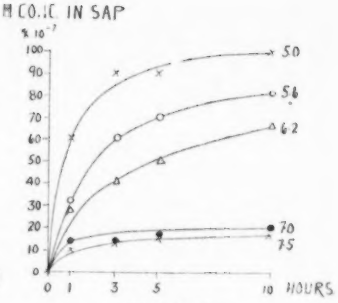

FIGURE 4.

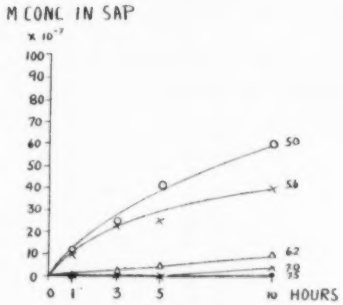

FIGURE 5.

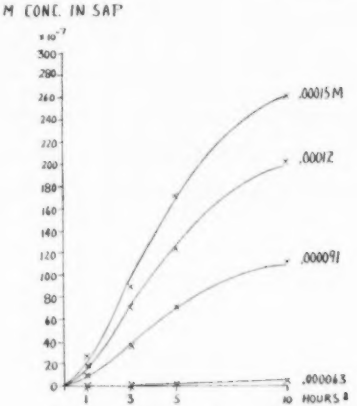

FIGURE 6.

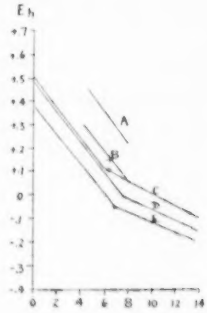

FIGURE 7.

TABLE 3

Curves showing agreement between observed (crosses) and calculated (smoothed curves) values for rate of penetration of 2, 6,-dibromo phenol indophenol into the sap of *Valoria* when $y = a\left(1 - \frac{k_1}{k_1 - k_2} e^{-k_2 t} + \frac{k_2}{k_1 - k_2} e^{-k_1 t}\right)$: Abscissae represent time in hours; ordinates, M concentrations of dye in the sap when multiplied by 10^{-7}; pH of external solution, 5.4; M concentration of external solution no. 1, 0.00015: no. 2, 0.00012: no. 3, 0.000091: no. 4, 0.000063. Temperature, 24.5 + 0.5°C.

t	$k_1 t$	$k_2 t$	$e^{-k_2 t}$	$\frac{k_1}{k_1 - k_2} e^{-k_2 t}$	$e^{-k_1 t}$	$\frac{k_2}{k_1 - k_2} e^{-k_1 t}$	$y/a =$	y, when $a =$ 300	y, when $a =$ 230	y, when $a =$ 125	OBSERVED M CONCENTRATION OF DYE IN SAP			
											No. 1	No. 2	No. 3	No. 4
1	0.25	0.6	0.5488	−0.395	0.7788	−1.323	0.072	21	16.5	9	25×10^{-7}	18×10^{-7}	10×10^{-7}	0
3	0.75	1.8	0.1653	−0.119	0.4723	−0.8029	0.317	95	72.5	39	90×10^{-7}	70×10^{-7}	36×10^{-7}	0
5	1.25	3.0	0.0497	−0.035	0.2865	−0.4870	0.548	164	126.0	69	170×10^{-7}	122×10^{-7}	70×10^{-7}	0.55×10^{-7}
10	2.5	6.0	0.0024	−0.0017	0.0820	−0.394	0.862	260	199.0	108	260×10^{-7}	200×10^{-7}	110×10^{-7}	1.6×10^{-7}

tion of 2,-6,- dibromo phenol indophenol into the sap of *Valonia* when the external pH was changed and the sap had been made more acid (5.2) by placing the cells in sea water containing CO_2, or more alkaline (pH 9.0) by placing the plants in sea water containing NH_4Cl before exposure to the dye. The abscissae represent time in hours, the ordinates, M concentration of dye in the sap when multiplied by 10^{-7}. The data are found in table 2.

These curves do not fit any of the simpler kinetic equation curves, and they would not be expected to, because the changing concentration of CO_2 and NH_3 in the sap and also, according to our hypothesis, in the contiguous layer of solution, introduce several complicating factors whose effect it is difficult to evaluate.

d. Analysis of effects of change in concentrations of dye. If the concentration of the external solution was changed while the pH values of both external and internal solutions were kept constant, results were obtained such as those shown in table 3 and figure 6. The time curves show the penetration of the dye into the sap, the abscissae representing time in hours, and the ordinates, molecular concentrations of dye in the sap. The concentrations of the external solutions were 0.00015, 0.00012, 0.000091 and 0.000063 M. At less than the last concentration, no appreciable amount of dye gets into the sap in ten hours at the temperature which was used (24.5°C. with a variation of less than one-half degree during the ten hours). Each point on the curve represents the average of 17 to 20 experiments. The pH of the external solution was 5.4; that of the sap normal, (6.4) with a gradual increase in acidity caused by the acidity of the external solution.

If the individual values of k, the velocity constant, are calculated from these data according to the equation of a bimolecular reaction there is a progressive rise in their values as time continues. The curves are convex to the axis of abscissae at the start, thus suggesting the presence of consecutive reactions, and for this reason the writer has tried to fit the formula for two consecutive unimolecular reactions (16) to the data. There is a close agreement as is seen from the curves, in which the crosses represent observed values, and the smooth curves are graphs of the equation:

$$y = a\left(1 - \frac{k_1}{k_1 - k_2}\, e^{-k_2 t} + \frac{k_2}{k_1 - k_2}\, e^{-k_1 t}\right)^{5}$$

where k_1 and k_2 have been assigned values of 0.25 and 0.6 respectively; t = time in hours; y = proportionate concentration of dye in the sap at time, t; and a = concentration of dye in the sap at equilibrium (which was estimated by extrapolating the curves).

[5] Mellor, *Higher mathematics for students of chemistry and physics.* Longmans Green Co., 1919, page 435. There is an error in the equation (8) as solved in Mellor which has been corrected by the writer in the equation used above.

It is important to study reactions under different conditions so that the true character of the reaction may be determined. When there are several reactions, and one is relatively very slow, the course of the complete reaction and its apparent order are fixed by the slow reaction and the fast reactions usually escape notice altogether. When the experimental conditions are changed, e.g., by changing the temperature or the concentrations, the velocity of one of the fast reactions may be so much reduced as to affect the character of the curve. Some such mechanism may account for the changes in the kind of curve found when different variables are introduced into the experiment with 2,-6,- dibromo phenol indophenol. It would have been interesting to try low concentrations at low temperature, but it was not practicable to procure a low temperature at the season of the year in which these experiments were done, and high concentrations at high temperatures were too toxic. It is hoped that further experiments will help to clear up some of these problems. It must be recognized that more complex series of related processes may actually be involved, but as only the more simple cases are susceptible to mathematical analysis, it is not possible to test more than a few hypotheses. None of these fit the observed data as well as that of two consecutive unimolecular reactions.

2. Results with other dyes. The curves in figure 7 represent the characteristic potentials of four dyes at various pH values (abscissae) and E_h values (ordinates) and their relative positions on the E_h scale. Curves *A* and *B* represent 2,-6,- dibromo phenol indophenol when the ratios of $\frac{[S_r]}{[S_o]}$ are $\frac{0.1}{99.9}$ and $\frac{99.9}{0.1}$ respectively. The former condition approximately obtains in the external solution, the latter in the sap. Curves *C*, *D* and *E* represent methylene blue, K-indigo tetrasulphonate, and K-indigo disulphonate respectively when the ratio of $\frac{[S_o]}{[S_r]}$ is $\frac{99.9}{0.1}$ the condition obtaining in the external solution, and in the sap in the case of curve *C*. These curves are drawn at 0.09 volt greater than Clark's curves for E_o'. These values may be calculated from the equation

$$E_h = E'_o - 0.03006 \log \frac{[S_r]}{[S_o]} + 0.03006 \log [Ka_1[H^+] + [H^+]^2] \quad (17)$$

in which E_o' is the electrode potential when the ratio of oxidant to reductant is 50:50; $[S_r]$, the amount of reduced dye; $[S_o]$, the amount of oxidized dye; Ka_1, the first acid dissociation constant of the reductant; and $[H^+]$ the hydrion concentration of the solution.

When $\frac{[S_r]}{[S_o]}$ is kept constant, the E_h will vary with the pH alone, and will then be a linear function of the pH so long as k_a (the acid dissociation

constant of the reductant) is small compared with $[H^+]$. Therefore we can draw a line parallel to the 50:50 curve at 0.09 volt above it which will indicate the oxidation-reduction potential of a solution in which the ratio of oxidant to reductant is 99.9 to 0.1.

The results of studies on the penetration of these dyes into the sap are interesting.

When K-indigo disulphonate, the lowest curve in figure 7, was used, no penetration into the sap could be detected by the method used. It is suggestive to note that the characteristic potential of indigo disulphonate is exceedingly low as compared with the other dyes.

It is also suggestive that this dye has the property of becoming more coarsely dispersed in the presence of salts. It may be that this is what happens in the sea water or in the protoplasm of the cell, and that this accounts for its non-appearance in the sap. These explanations are, however, all highly speculative.

K-indigo tetra sulphonate was found in the sap in a faint yellow form. No quantitative measurements of this dye could be made by the colorimetric method. The yellow color may have been due either to a reduced form of the dye, or to a new product called isatin, which is an oxidized form of indigo tetra-sulphonate. Since the yellow color could not be changed to blue by any of the ordinary methods of oxidation, it seems probable that the dye was not reduced but rather that isatin was formed. Since the position of this dye in the figure or in other words, its characteristic potential, is considerably lower than that of the indophenol dye, one might expect little or no reduction in the cell.

Furthermore, the yellow compound was found in the sap only when the external solution was alkaline or when the cell sap had been made alkaline by treating the cell with NH_4Cl previous to placing it in the dye.

Methylene blue, the dye which is nearest indophenol on the figure, was found to penetrate into the sap only in an oxidized form at all external and internal pH values used, and no evidence of reduction could be found by the colorimetric method. Also no differences in amounts of dye in the sap could be noted at different pH values of external solution.

Furthermore, no such difference between the amount of dye in the sap and that in the external solution was found as in the case of 2,-6,- dibromo phenol indophenol. In fact, the amount of dye in the sap was within three hours practically the same as that in the outside solution, after which there was little if any increase, showing that an equilibrium had been reached. Since methylene blue is a very strong base, it is completely dissociated at all pH values used in these experiments. In other words, all the dye present is in a dissociated condition.

It may be objected that slight amounts of undissociated dye are always present in the external solution and that it is these which penetrate. But

these must vary in amount according to the equation for the dissociation of methylene blue:

$$K = \frac{(Me)'\ (OH)}{MeOH}$$

in which (Me)′ indicates the amount of the cation of methylene blue, and MeOH the amount of undissociated dye.

If, however, (Me)′ is practically constant, $\frac{K}{(Me)}$ should be constant and should equal $\frac{OH'}{MeOH}$. Therefore MeOH is inversely proportional to H and at pH 9 the amount of dye in the sap should be 10,000 times the amount at pH 5, which is of course not the case in the experiments.

It may also be suggested that the oxidized dye changes to a reduced form before it enters the protoplasm. Since the reduced dye is a much weaker base whose first dissociation constant is 1.4×10^{-8} much of it would be present in the undissociated form. Provided the assumption holds good that undissociated dye only enters the cell there would be a hundredfold difference in the amount of dye found in the sap at pH's 5 and 9. This once more is not the case.

Therefore this is further evidence against the hypothesis lately advocated by Osterhout (18) that only undissociated molecules can penetrate living cells.

Another example has been commented upon by the writer in a paper (in course of publication) discussing previously published results upon the penetration of arsenic (5).

There may be some hitherto unknown relationship between

I. The penetration of a reduced substance whether reduced by the protoplasm or already reduced, and its ability to penetrate in the undissociated form.

II. The penetration of an oxidized substance as such, and its ability to penetrate in the dissociated form.

Examples of the former are 2,- 6,- dibromo phenol indophenol, and H_2S, and an example of the latter is methylene blue.

In other words, when a substance has characteristics like those of the reduced dye it can only penetrate in proportion to the extent of reduction and undissociation while another type of substance like an oxidized dye can penetrate when it is ionized. This may explain why in some cases the ions enter and in other cases the undissociated molecules.

Summarizing these results, it may be said that the cell cannot reduce methylene blue (at least not to a great extent), but that the dye penetrates in the oxidized form. The same is true of K-indigo tetra sulphonate, but K-indigo disulphonate is not found in the sap.

Voegtlin et al (19) found that mammalian tissues reduce all of these dyes *in vitro* under anaerobic conditions also in the order mentioned above, but *in vivo* the dyes of class II and III are reduced only after a long time. More work is, however, needed on other organisms to determine whether this is a fundamental law of all tissues, as it seems to be. One might conclude from these data that mammalian tissues have a somewhat lower oxidation-reduction potential than *Valonia*.

3. Determination of E_h and rH of Valonia. We can estimate the probable limits of the E_h value of the protoplasm of *Valonia* if we assume that the amount of dye penetrating into the sap is a constant times the per cent reduced in the protoplasm (as is discussed in an earlier part of this paper), and furthermore, if we assume that the pH of the protoplasm should reasonably fall somewhere between 4 and 8. Then the E_h of the protoplasm, or region of the protoplasm which is concerned with reduction, should fall somewhere along curve *A*, or between +0.21 and +0.48.

In dealing with the protoplasm, one must not forget that it is a polyphase heterogeneous system of various physical-chemical characteristics. One can therefore not speak of the oxidation-reduction potential of the protoplasm as a whole without this reservation.

The calculations for E_h and rH of the sap are delimited by the fact that the expressed sap does not reduce certain dilute concentrations of 2,- 6,-dibromo phenol indophenol while the sap intact contains this dye in a reduced form. This means that the sap is not "poised" (the term being used for oxidation potentials much in the same way as "buffer" is used for H ion concentrations), and that therefore it assumes the E_h of the substance placed in it, which in this case is the penetrating dye. Although further experimentation is necessary to obtain the E_h and rH of the sap, nevertheless because of such remarkable agreement with the work of others the calculations will be here included and must be considered only tentative.

Curve *B* represents the condition of the dye in the sap. The curve is drawn parallel to that of E_o' at 0.09 volt lower. At pH 6.4 (the pH of the sap), on this curve the value of E_h is 0.15 (marked by a cross). If we consider the dye 100 per cent reduced, this value would be infinity, but with the help of another indicator such as methylene blue we can further limit the bounds.

We know that methylene blue is found oxidized almost completely or nearly so, in the sap, and we shall therefore call the ratio of $\frac{[S_o]}{[S_r]}, \frac{99.9}{0.1}$ and draw the curve for this per cent of oxidation of methylene blue 0.09 volt higher than that for the 50:50 ratio (curve *C*). At pH 6.4, the value for E_h is 0.12. Therefore the oxidation reduction potential of the sap must be above 0.12 and not higher than 0.15. By the use of another indicator whose curve lay between these two, one could determine more exactly what the value is.

Having given the E_h of the sap of *Valonia* and the pH, we can obtain rH, or the logarithm of the reciprocal of the hydrogen pressure from figure 10 of Clark and Cohen (7) as between 16 and 18. This figure shows the relation between $\log \frac{1}{p}$ or rH and E_h, and follows the equation,

$$E_h = -0.06\ pH$$

in which the E_h becomes more negative by 0.06 volt for each unit increase in pH.

One can calculate from the data and assumptions already presented for the E_h and pH of the protoplasm or region of protoplasm dealing with oxidation-reduction that the rH would be likely to fall between 14 and 27. Since the region of oxygen overvoltage begins at 27.3 it is more likely that the rH of the protoplasm would be considerably lower than this. If we knew the rH, we could exactly determine the pH of the protoplasm and conversely, if we knew the pH, we could exactly calculate the rH.

In this connection it is interesting to make a comparison with the work of Needham and Needham (20) on *Amoeba proteus*. They found the rH of the cell-interior to be between 17 and 19, and the E_h to be +0.097 (subject to certain assumptions). This E_h value is slightly lower than that found by the writer for *Valonia* which was +0.12 to +0.15 tentatively for sap and between +0.21 and +0.48 for protoplasm (subject also to certain assumptions); the rH for the sap was between 16 and 18.

It is quite striking that the tentative value for rH of the sap of *Valonia* (between 16 and 18) and of the cell-interior of *Amoeba proteus* (between 17 and 19) should be so nearly alike, since they are such widely separated forms and since the methods of experimentation were so different. It may be that this value, when much more accurate methods of experimentation are used is the same for all forms; or that there is a slightly lower oxidation potential in animals than in plants. In the latter case, this would be one of the essential quantitative differences between these two forms of life. But more work is of course necessary on other forms before definite statements can be made.

The oxidation-reduction indicators with their exact physical-chemical measurements have opened up an entirely new field for the biologist and offer possibilities of approach in quantitative analysis which have not yet been developed. We may find that many of the phenomena which have been considered to be effects of pH are also attributable to E_h; and that in the future this factor must be considered in studying reasons for penetration of substances into cells.

SUMMARY

The penetration into the sap of *Valonia* of several oxidation-reduction indicators was studied and the following results obtained:

1. Experiments with 2,-6,- dibromo phenol indophenol.

Only reduced dye enters the sap.

The penetration of the dye into the sap follows the course of a bimolecular reaction curve.

The amount of dye in the sap at equilibrium is proportional to the amount of undissociated dye in the external solution.

When the sap is made more acid than normal, there is more dye present; and less dye when the sap is made more alkaline.

At higher temperatures and lower concentrations the curves follow a course like that for two consecutive unimolecular reactions.

2. Experiments with other oxidation-reduction indicators.

Methylene blue was found to penetrate into the sap in an oxidized form. The amount present did not vary with external nor internal changes in pH values from 5 to 9.

K-indigo tetrasulphonate was found to penetrate into the sap in a yellow form.

K-indigo disulphonate could not be found in the sap by means of the methods used.

Results with these indicators do not support the hypothesis of Osterhout that only undissociated molecules enter living cells.

3. The oxidation-reduction potential of the sap was tentatively found to be between rH 16 and 18; that of the protoplasm was less exactly defined.

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ESTIMATION OF THE SURFACE AREA OF THE WHITE RAT

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Received for publication January 11, 1926

The pronounced effect of the size of an animal upon its basal heat production renders it necessary in experiments concerned with the influence of other factors to eliminate the influence of differences in the size of experimental animals insofar as this may be possible. The most common method of eliminating the effect of size of animal upon any physiological function is to refer this function to a unit of body weight. However, for animals differing widely in size, the basal heat production per unit of body weight is not even approximately constant under the same conditions, but increases with decreasing body weight. Another system of eliminating the effect of size of body upon basal heat production is to compute the latter per unit of body surface. This system is commonly associated with the name of Rubner and was apparently based upon purely physical considerations, namely, the law of cooling bodies of Newton.

The "surface area law" of Rubner has been attacked from many angles, on the basis both of theory and of fact. Its literal application to animals is implicitly based upon a series of questionable assumptions: first, that the temperature of all parts of an animal's skin is the same; second, that the radiating capacity of the body surfaces of different animals is the same; and third, that the radiating capacity of the surface of an individual animal does not change with those changing conditions of the animal and its environment which are not incompatible with a determination of the basal metabolism. The law thus neglects obvious anatomical and physiological differences among animals, and in its original form, at least, implies that heat emission is the only factor regulating basal heat production.

However, its advocates have recently rescinded this literal interpretation of the surface area law, preferring to believe merely that the surface area of an animal is more closely related to its content of protoplasmic material, i.e., its heat-producing tissue, than is the body weight or any other available biometric measurement. In giving this interpretation to the surface area law, it becomes entirely empirical in nature, while its application to different species of animals differing widely in size implies a difference in anatomical composition so wide as to be absurd. For example, a man of average height (173 cm.) and weight (70 kgm.) weighs 350 times as much

TABLE 1

The surface area of rats of different ages and weights

RAT NUMBER	LIVE WEIGHT	AGE	SEX	LENGTH FROM WITHERS TO TAIL	SKIN AREA	MEEH CONSTANT	PER CENT DEVIATION OF CONSTANT FROM AVERAGE
	grams	*days*		*cm.*	*sq. cm.*		
1	25	21	m	5.5	111	12.98	+14.3
2	26	21	f	6.0	104	11.96	+5.3
3	41	21	f	6.5	140	11.89	+4.7
4	46	21	f	7.0	146	11.45	+0.8
5	54	30	f	7.0	177	12.39	+9.1
6	58	30	f	7.5	185	12.41	+9.2
7	60	35	m	8.0	175	11.42	+0.5
8	64	35	f	8.0	181	11.38	+0.2
9	67	35	f	7.5	177	10.73	−5.5
10	69	35	f	7.5	201	11.95	+5.2
11	70	35	m	8.0	172	10 19	−10.3
12	71	35	m	7.5	191	11.20	−1.4
13	71	35	f	8.5	201	11.72	+3.2
14	76	49	f	8.5	217	12.10	+6.5
15	78	40	f	8.5	212	11.67	+2.7
16	82	35	f	8.5	229	12.14	+6.9
17	98	60	f	9.5	240	11.29	−0.6
18	108	40	f	9.0	257	11.33	−0.2
19	109	40	m	9.5	249	10.91	−4.0
20	127	90	f	10.0	242	9.58	−15.7
21	127	90	f	11.0	272	10.80	−4.9
22	131	60	m	10.5	291	11.28	−0.7
23	133	90	f	11.5	282	10.86	−4.4
24	139	90	f	11.5	295	10.99	−3.3
25	139	75	f	11.0	299	11.18	−1.6
26	140	90	f	12.0	264	9.80	−13.7
27	162	90	m	11.0	338	11.40	+0.3
28	175		f	11.0	342	10.93	−3.8
29	190	90	m	12.0	374	11.35	−0.1
30	190	105	f	11.0	379	11.47	+1.0
31	190	105	m	12.0	406	12.31	+8.4
32	190	105	f	12.0	375	11.38	+0.2
33	195		f	11.5	371	11.06	−2.6
34	200	120	m	12.0	389	11.40	+0 3
35	206	120	m	13.0	387	11.10	−2.3
36	206	135	m	12.0	390	11.21	−1.3
37	208		f	12.0	375	10.68	−6.0
38	211	120	m	13.0	405	11.46	+0.9
39	216	120	m	13.5	416	11.56	+1.8
40	216	135	m	12.5	397	11.03	−2.9
41	217	90	m	13.0	410	11.35	−0.1
42	217	120	m	12.5	413	11.47	+1.0
43	222	120	f	13.0	420	11.46	+0.9
44	228		f	13.0	445	11.92	+4.9

TABLE 1—*Concluded*

RAT NUMBER	LIVE WEIGHT	AGE	SEX	LENGTH FROM WITHERS TO TAIL	SKIN AREA	MEEH CONSTANT	PER CENT DEVIATION OF CONSTANT FROM AVERAGE
	grams	*days*		*cm.*	*sq. cm.*		
45	229	120	m	13.5	429	11.46	+0.9
46	230		m	13.0	389	10.39	−8.5
47	231	120	m	13.5	423	11.24	−1.1
48	233	120	m	13.0	443	11.70	+3.0
49	237	120	f	13.0	423	11.07	−2.6
50	249		m	12.5	458	11.57	+1.8
51	250	135	m	13.5	448	11.29	−0.6
52	252		m	13.5	440	11.05	−2.5
53	253		m	13.0	460	11.53	+1.5
54	264	135	m	14.0	475	11.54	+1.6
55	289		m	14.0	481	11.00	−3.2
56	297		m	14.5	564	12.64	+11.3
57	305		m	13.5	521	11.52	+1.4
58	314		m	13.5	521	11.28	−0.5
59	315		m	14.0	540	11.66	+2.6
60	340		m	14.0	552	11.33	−0.3
61	345		m	14.0	525	10.69	−5.9
62	461		m	17.0	673	11.29	−0.6
Average						11.36	

as a 200 gram rat, although his surface area is only 47 times that of the rat. According to this latest interpretation of the surface area law, the man should contain only 9.4 kgm. (0.2 × 47), or 13.4 per cent, of protoplasmic material, on the extreme assumption that the rat consists entirely of active cellular tissue. In its application to different species of animals, at least, it appears that the literal interpretation of the law, if such it proves to be, is the more plausible of the two.

However, the value of any method of computing the basal heat production of an animal to some unit of size does not depend upon theoretical considerations, nor is it valueless because it does not yield constant results over wide ranges of condition. In spite of its theoretical weakness, the computation of basal metabolism to the unit of body surface seems at present the most satisfactory method available of equalizing experimental results for differences in the size of experimental animals.

The most ambitious attempt to demonstrate the general applicability of Rubner's surface area law is that of E. Voit (1), who has compiled a table giving the heat production of resting animals varying in size from a 2 kgm. hen to a 441 kgm. horse. When computed to a kilogram of body weight, the heat production of these animals varied from 11.3 calories to 75.1 calories per day, while when computed to the square meter of body

surface the variation ranged only from 776 to 1078 calories per day. The significance of this table, however, is seriously impaired by the fact that the activity of the animals was not carefully controlled in the determination of basal metabolism, and that the estimations of body surface cannot be considered accurate. In these investigations the well-known formula of Meeh was used. No great confidence, however, can be felt in the accuracy of the constants used in the Meeh formula, either because the constant was determined on only one or two individuals in the species, or because the constant determined for one species was arbitrarily assumed to be sufficiently accurate for another. For example, the constant used for the horse was determined on one animal by Hecker (2) and was applied not only to this species, but also to the pig. Similarly, the constant used for the hen was actually obtained upon a goose. Evidently an accurate estimation of body surface is an important requisite to the satisfactory application of this method of expressing the basal metabolism of animals.

In the following paper the results of experiments to determine the effect of various factors other than size upon the basal heat production of the white rat will be reported. To permit the computation of these results to the square meter of body surface, it seemed necessary to devise a method by which the surface area of a rat could be estimated with sufficient accuracy from its weight, or possibly from its weight and some linear body measurement. With this purpose in view the experiment reported in this paper was undertaken.

Sixty-two rats ranging in weight from 25 to 461 grams were killed and their skin areas determined by stretching the skins on paper, outlining them and measuring the inclosed areas with a planimeter. The areas thus obtained represented the entire surface area of the rat except for the ears. At the same time a body length measurement was taken on each carcass from the root of the tail to the withers. The results of the determinations are given in the table. In the second column from the right the Meeh constant, k, is given for each rat as determined from the following equation:

$$S = k\, W^{\frac{2}{3}}$$

in which S equals the skin area in square centimeters and W equals the weight in grams.

The average Meeh constant for this group of rats is 11.36 ± 0.05. The standard deviation of the individual constants is 0.589 and the coefficient of variation, 5.19 per cent. In the last column to the right in the table is given the percentage deviation of each individual value of k from the average value of k for the group. These percentages would represent the error in estimating the individual surface areas from the body weights by the use of the average value of k for the entire group of 62 rats. The largest percentage error is 15.7. However, 74.2 per cent of the 62 individ-

ual values of k were no more than 5 per cent from the average value of 11.36, while only 8.1 per cent were greater than 10 per cent from it. The average percentage deviation was 3.61. There is no tendency evident for the individual values of k either to progressively increase or to progressively decrease from the smaller to the larger rats.

Several attempts to increase the accuracy of the Meeh formula by introducing into it the body length measurement taken on the rats proved unsuccessful. It was therefore decided to use the unmodified Meeh formula with a constant equal to 11.36 in estimating the body surface of these animals. It may be pointed out that no significant sex difference in the size of this constant is evident from the data compiled in the table. For the 34 male rats in this table the average value of k was 11.38, while for the 28 female rats the average value was 11.34.

The Meeh constant obtained in this investigation is considerably higher than the constant given by Rubner (3) for the rat, namely, 9.1. No information as to the number of animals used in obtaining this constant has been found.

SUMMARY

In this investigation the surface area of 62 rats varying from 25 to 461 grams in weight was determined. The average Meeh constant for these 62 determinations was 11.36. The average deviation of the individual values of k in the Meeh formula from the average value of k was 3.61 per cent. Approximately three-fourths of the individual values of k were within 5 per cent of the average, while only 8 per cent of the individual values of k were greater than 10 per cent from the average.

The male and female rats gave practically identical values of k in the Meeh formula.

Attempts to increase the accuracy of the Meeh formula for estimating the body surface of rats by introducing into it some function of a body length measurement were not successful.

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EFFECT OF EXCESSIVE AMOUNTS OF VITAMIN B ON THE BASAL METABOLISM OF RATS OF DIFFERENT AGES

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Received for publication January 11, 1926

Investigations concerned with the vitamin requirements of animals may profitably be extended to a consideration of the possibility that the ingestion of amounts of vitamins greater than the apparent minimum requirements may exert beneficial effects upon an animal, such as an increased resistance to disease or a general increase in physiological efficiency or "vitality." While no satisfactory measure of an animal's vitality can be said to have been discovered, it is becoming increasingly evident that the intensity of the basal metabolism varies directly with an animal's physiological condition. For example, athletes are known to have a higher basal metabolism than untrained persons, and men a higher basal metabolism than women; debilitating diseases are known to lower the basal metabolism, while convalescence is accompanied by an increased metabolism. In the same connection, Lusk has shown (1) that continued confinement in a cage lowers the basal metabolism of a dog as much as 25 per cent, although the body weight remains unchanged. It was the purpose of the experiment here reported, therefore, to determine the effect of excessive amounts of vitamin B upon the basal metabolism of animals. The white rat was chosen as the experimental subject.

Several investigations have indicated that vitamin B is intimately concerned in tissue respiration. In 1921 Hess showed that there was a marked difference between the surviving tissues of healthy and polyneuritic pigeons with respect to their ability to reduce m-dinitrobenzene to m-phenylhydroxylamine. The oxidative processes were found to be greatly reduced in the tissues of the polyneuritic pigeons examined as compared with healthy pigeons or pigeons cured of polyneuritic symptoms (2). In conformity with Hess's results, Fleisch (3) has shown that in birds suffering from vitamin B deficiency, the difference between the oxygen content of arterial and venous blood is only about one-half the normal difference, although the arterial blood is even more completely saturated with oxygen in this condition than in normal animals. The same condition was noted in birds suffering from cyanide poisoning and the comparable findings in avitaminosis and KCN poisoning are taken to support

the view that in the former condition as in the latter, there is a reduction of the oxidative processes in the tissues.

The experimental results just reviewed are in agreement with the experiments of Abderhalden who showed that the oxygen consumption of the tissues of polyneuritic pigeons was much lower than that of the tissues of normal pigeons (4), and that this low cell respiration can be promptly raised by the addition to the oxidizing mixture of an alcoholic extract of fresh or autolyzed yeast. Abderhalden has also shown (5) that in such dystrophic pigeons the cysteine content of the organs is diminished from normal, a fact of great significance in relation to the finding of Hopkins that glutathione, a peptide containing cysteine, is undoubtedly concerned in the oxidative processes in the tissues. Rohr (6) has pointed out the close correlation between the vitamin B content of different organs and their rate of respiration.

Although these results on surviving tissues point to an intimate relation between the vitamin B intake of animals and the intensity of oxidation processes in their tissues, other experiments indicate that this effect may be indirect. It appears that although the basal metabolism of rats suffering from a deficiency of vitamin B may be greatly lowered from normal, this result is directly dependent upon an insufficient intake of food, characteristic of this type of avitaminosis, since control animals receiving adequate amounts of the vitamin likewise show diminution in basal metabolism if their food intake is restricted to the intake of the rats on the incomplete diet. This has been shown clearly by Gulick's careful investigations along these lines (7). Essentially the same results have been reported by Deuel and Weiss in experiments on the dog (8). These results suggest that the reduction in tissue respiration noted in avitaminotic pigeons may be an effect of inanition or undernutrition. On the other hand, Ahlgren's experiments (9) show that rats and pigeons differ in their reaction to vitamin B deficiency, pigeons showing a reduction in tissue respiration and rats showing none.

METHODS OF INVESTIGATION. Five litters of rats were used in this investigation. Their ages at the time that they were put upon experimental feeding were as follows:

Litter A 41 days of age
Litter B 32 days of age
Litter C 32 days of age
Litter D 30 days of age
Litter E 32 days of age

They were all placed upon a basal ration containing 18 per cent of casein (a high grade commercial product), 4 per cent of the Osborne and Mendel salt mixture, 8 per cent of butter, 2 per cent of cod liver oil and 68 per cent of corn starch. Each rat received in addition to this ration regulated

amounts of yeast vitamin (Harris). Within each litter the different rats were given amounts of this preparation varying from 10 mgm. to 160 mgm. daily. Except for the smallest amount of vitamin concentrate, the amounts given may be considered greater than the minimum requirements. Thus, Osborne and Mendel (10) have shown that a daily intake of 200 mgm. of dried yeast is sufficient to promote normal growth to maturity in the albino rat, while Osborne and Wakeman (11) have found that the greater part of the vitamin B contained in yeast is concentrated in their fraction II which represents about 6 per cent of the yeast solids. According to these experimental results 15 mgm. of fraction II (yeast vitamin, Harris) daily should promote normal and complete growth in the rat. The food intake and the body weight of each rat were determined weekly.

At least once and generally twice during the experiment each rat was subjected to a basal metabolism determination. The method used in this determination was essentially that described by Haldane (12) in 1892. This is a gravimetric method for the determination of the respiratory exchange of small animals. The principle of the method is briefly as follows. The animal is confined in a small air-tight chamber which is ventilated by a water pump. The incoming air is rendered water- and carbon-dioxide-free by passage through a series of bottles containing soda lime for the absorption of carbon dioxide and sulfuric acid for the absorption of water. The air leaving the chamber is passed through a similar chain of absorption bottles. The carbon dioxide production is measured by the gain in weight of the soda lime bottle and the sulfuric acid bottle into which it leads in the outgoing chain. The oxygen consumption is measured by the total increase in weight of the animal and animal chamber and of all of the absorption bottles in the outgoing chain, since the only difference between the air entering the animal chamber and the air leaving the last of the second train of absorption bottles is the difference in oxygen content caused by the respiration of the experimental animal. From the oxygen consumption and the respiratory quotient, the heat production of the animal was computed, neglecting the catabolism of protein (13). The error thus introduced is known to be small because the protein calories produced after a short fast are known to be but a small fraction of the total calories, but mainly because the calorific value of a liter of oxygen used in the combustion of protein is so nearly equal to the calorific value of a liter of oxygen used in the combustion of a mixture of fat and carbohydrate possessing an R. Q. equal to that of protein.

After considerable experience with this gravimetric method, particularly in devising adequate means of checking the efficiency of the several absorption bottles and of detecting and remedying leaks in the apparatus, we have found that the method is capable of great accuracy, while at the same time it involves very little technical skill or continuous attention.

Its usefulness is restricted by the fact that an accurate determination of the oxygen consumption can be made usually only in periods of several hours' duration, since the weight of the animal chamber cannot be accurately taken if the animal is at all restless. In the routine use of the method the efficiency of each sulfuric acid bottle should be checked by placing immediately in front of it a smaller duplicate absorption bottle, which should remain practically constant in weight throughout the test. A similar method of checking the efficiency of the carbon dioxide absorption bottles in the outgoing train is also necessary. After the animal is sealed into the animal chamber with paraffin it is advisable to test the air-tightness of the chamber by means of a manometer.

In his original method, Haldane used water-absorption bottles filled with small pieces of pumice saturated with sulfuric acid, the purpose of this arrangement being to permit a pressure in the animal chamber only slightly less than atmospheric. We have found it much more convenient, however, to use concentrated sulfuric acid only in these bottles. The small reduction in pressure in the animal chamber occasioned by this change cannot be considered to exert any effect upon the basal metabolism of the animal (14).

Although the accurate determination of oxygen consumption in this apparatus usually requires experimental periods of several hours' duration, the carbon dioxide production can be determined accurately in short periods. The method of using the apparatus in determining the basal metabolism of rats in this experiment was to fast the animal for approximately 24 hours and then to subject it to a period of 4 to 6 hours in the apparatus. For this entire period, the respiratory quotient was determined from the oxygen consumption and the carbon dioxide production. However, the heat production throughout this period could not be considered basal, since the activity of the animal in the chamber could not be controlled. Therefore, the carbon dioxide production in short periods of absolute rest was determined at convenient intervals in the longer period of 4 to 6 hours. This was rendered possible by a second set of carbon dioxide absorption bottles in the outgoing train, through which the air could be diverted at will by simply turning a stopcock. These sub-periods could be chosen at times when the animal was seen to be at absolute rest. If the animal became restless, in the course of such a period, the air current was diverted through the other set of absorption bottles until it again became quiet and at least 5 minutes had elapsed to permit of a thorough washing out of the chamber (a glass bottle just large enough to contain the animal comfortably) from the increased amounts of carbon dioxide resulting from the muscular activity of the rat. It is important that the rate of ventilation be constant throughout these sub-periods and that it be approximately equal to the ventilation rate for the long periods. In these

experiments the ventilation rate varied from 600 cc. to 1000 cc. per minute depending upon the size of rat. The sub-periods generally lasted 20 to 25 minutes. From the amounts of carbon dioxide determined during these periods of absolute rest and from the respiratory quotient of the long period of 4 to 6 hours, the basal heat production of the rat could be computed. As the experiment progressed, it became evident that the respiratory quotient of the fasted rats was so nearly constant, that an average value of 0.75 could be assumed with a minimum of error. Therefore, in the later tests the determination of oxygen consumption was dispensed with.

In determinations of the basal metabolism of animals it is important that the temperature within the animal chamber shall not become so low that the factors of chemical regulation of body temperature begin to operate; in other words, that the temperature in the animal chamber shall always

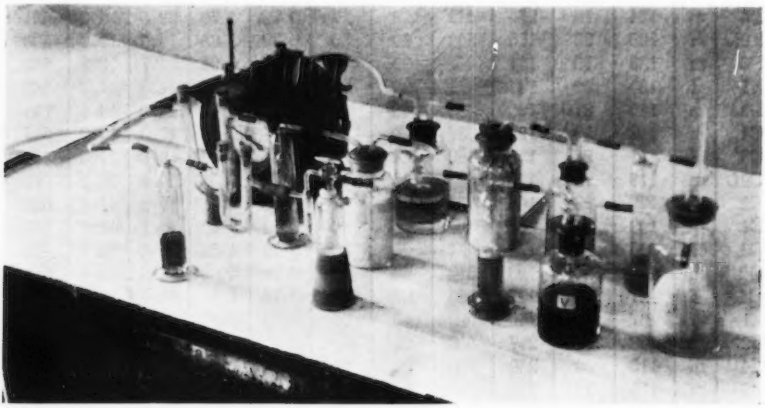

Fig. 1

be above the critical temperature. The critical temperature of the rat according to Goto (15) is about 28°C. Throughout these determinations, therefore, the temperature of the animal chamber was not allowed to fall below 28° nor to rise above 31°. The temperature was controlled by means of a bunsen burner kept at a suitable distance from the chamber. The modified Haldane apparatus used in this investigation is shown in the accompanying photograph.

EXPERIMENTAL RESULTS. The results of the basal metabolism determinations together with information concerning the size, sex and age of the experimental animals and the amount of vitamin supplement consumed daily are given in table 1. The body surface of the rats was estimated from their weight by means of the Meeh formula with a constant of 11.36 (see preceding article).

TABLE 1

Details of the basal metabolism determinations

RAT NUMBER	SEX	AGE	LENGTH OF FAST	BODY WEIGHT	BODY SURFACE	DAILY VITAMIN SUPPLEMENT	RESULTS OF RESPIRATION EXPERIMENT					
							Length	R.Q.	O_2 per day	Heat produced per day		
										Total	Per 100 grams body weight	Per square meter body surface
		days	*hrs.*	*gms.*	*sq. cm.*	*mgm.*	*min.*		*liters*	*cals.*	*cals.*	*cals*
B1	f	59	18	84	216	20	60	0.82	2.74	13.1	15.6	609
A4	m	91	18	151	322	120	30	0.76	3.79	18.0	11.9	559
A3	m	93	18	150	320	80	30	0.76	3.64	17.3	11.5	540
A2	f	96	18	128	288	40	30	0.72	3.41	16.0	12.5	556
B4	m	134	18	234	432	120	17	0.78	4.72	22.5	9.6	521
B2L	f	135	22	170	349	40	30	0.73	3.96	18.6	10.9	533
B3	m	136	18	191	377	80	30	0.74	5.23	24.7	12.9	655
B1	f	139	22	187	371	20	30	0.69*	4.61	21.6	11.5	583
B2	m	140	21	198	386	40	30	0.72	6.15	28.9	14.6	748
C4	m	110	20	199	387	120	30	0.76	5.68	27.0	13.6	698
C3	f	111	23	171	350	80	30	0.73	4.10	19.3	11.3	551
C2L	m	118	22	196	383	40	26	0.74	5.48	25.9	13.2	676
C2	f	122	23	166	364	40	17	0.72	4.08	19.2	11.6	527
C1	m	123	23	202	391	20	25	0.81	5.42	26.1	12.9	667
D1	f	120	22	172	351	20	30	0.71	4.52	21.2	12.3	605
D2	m	121	24	223	418	40	24	0.76	5.64	26.8	12.0	641
D4	f	127	22	177	358	120	30	0.75	4.52	21.4	12.1	600
E1	f	107	22	150	320	10	30	0.74	3.79	17.9	11.9	560
E2	m	108	22	217	410	20	30	0.73	5.59	26.3	12.1	643
E4	m	113	23	240	439	80	20	0.75	4.12	19.5	8.1	444
E3	m	114	19	232	429	40	30	0.73	5.75	27.1	11.7	631
E5	f	115	23	188	373	160	28	0.71	5.38	25.2	13.4	676
B1	f	174	24	212	404	20	23	0.74	5.06	23.9	11.3	592
B2	m	175	20	238	436	40	30	0.74	6.01	28.4	11.9	651
B2L	f	176	23	187	371	40	22	0.73	5.01	23.6	12.6	636
B3	m	177	21	217	410	80	30	0.75	4.28	20.3	9.4	495
B4	m	188	24	277	483	120	24	0.75†	5.19	24.6	8.9	509
B4	m	189	53	273	478	120	24	0.75	5.43	25.7	9.4	538
C1	m	160	24	224	419	20	24	0.75	4.57	21.7	9.7	517
							15	0.75	5.42	25.7	11.4	618
C3	f	161	25	191	377	80	24	0.75	3.92	18.6	9.7	493
							24	0.75	3.92	18.6	9.7	493
C4	m	162	24	247	448	120	24	0.75	4.57	21.7	8.8	484
							24	0.75	4.54	21.5	8.7	480
C2	f	164	22	184	367	40	24	0.75	3.72	17.6	9.6	480
							24	0.75	4.25	20.1	10.9	549
C2L	m	165	20	227	423	40	24	0.75	5.84	27.7	12.2	654
							18	0.75	5.39	25.6	11.3	604

TABLE 1—*Concluded*

RAT NUMBER	SEX	AGE	LENGTH OF FAST	BODY WEIGHT	BODY SURFACE	DAILY VITAMIN SUPPLEMENT	RESULTS OF RESPIRATION EXPERIMENT					
							Length	R.Q.	O_2 per day	Heat produced per day		
										Total	Per 100 grams body weight	Per square meter body surface
		days	*hrs.*	*gms.*	*sq. cm.*	*mgm.*	*min.*		*liters*	*cals.*	*cals.*	*cals.*
D1	f	162	24	187	371	20	24	0.75	4.57	21.7	11.6	584
							18	0.75	4.30	20.4	10.9	549
D2	m	168	21	259	461	40	24	0.75	6.29	29.8	11.5	646
							24	0.75	6.04	28.6	11.0	621
D3	m	171	16	269	474	80	28	0.75	7.80	37.0	13.8	780
							24	0.75	7.18	34.0	12.6	718
D4	f	171	21	203	392	120	20	0.75	6.16	20.4	10.0	521
							24	0.75	6.01	20.0	9.8	509
E1	f	154	25	173	353	10	24	0.75	4.33	20.5	11.8	581
							24	0.75	4.25	20.1	11.6	571
E3	m	163	24	285	492	40	24	0.75	5.80	27.5	9.6	559
							24	0.75	5.88	27.9	9.8	568
E4	m	164	26	267	471	80	24	0.75	5.33	25.3	9.5	536
							24	0.75	5.68	26.9	10.1	572
E5	f	165	25	192	378	160	24	0.75	4.86	23.0	12.0	610
							24	0.75	4.65	22.0	11.5	583
E2	m	166	25	251	452	20	24	0.75	5.35	25.4	10.1	561
							24	0.75	5.19	24.6	9.8	544

* An R. Q. of 0.727 assumed in the calculations.

† In this and the following experiments, no determinations of the respiratory quotient were made. The average quotient of all preceding experiments, 0.748, was assumed.

Before discussing these results relative to the vitamin B intake, it seems necessary to determine what effect the age and sex of the rats had upon their basal metabolism. In table 2, therefore, the results are grouped according to age and sex. Except for those rats less than 100 days old and those more than 175 days old, the average basal heat production per square meter of body surface was greater for the males than for the females. The two extreme groups showing an opposite relation contained only one or two rats each. For all tests the basal metabolism of the male rats averaged 600 ± 11 calories per day per square meter of body surface, while that of the female rats averaged 571 ± 7 calories a difference of 29 ± 13 calories, or approximately 5 per cent. This average sex difference, while not highly significant with reference to its probable error, is almost identical with that found among human beings. The daily basal metabolism per 100 grams body weight does not show any consistent relation-

TABLE 2

The effect of age and sex upon the basal metabolism of rats

AGE GROUP	MALES					FEMALES				
	Rat	Age	Weight	Heat produced daily		Rat	Age	Weight	Heat produced daily	
				Per 100 grams body weight	Per square meter				Per 100 grams body weight	Per square meter
days		*days*	*gms.*	*cals.*	*cals.*		*days*	*gms.*	*cals.*	*cals.*
Less than 100	A4	91	151	11.9	559	B1	59	84	15.6	609
	A3	93	150	11.5	540	A2	96	128	12.5	556
Average.....		*92*	*150*	*11.7*	*549*		*77*	*106*	*14.0*	*582*
100–125	E2	108	217	12.1	643	E1	107	150	11.9	560
	C4	110	199	13.6	698	C3	111	171	11.3	551
	E4	113	240	8.1	444	E5	115	188	13.4	676
	E3	114	232	11.7	631	D1	120	172	12.3	605
	C2L	118	196	13.2	676	C2	122	166	11.6	527
	D2	121	223	12.0	641					
	C1	123	202	12.9	667					
Average.....		*115*	*216*	*11.9*	*629*		*115*	*169*	*12.1*	*584*
125–150	B4	134	234	9.6	521	D4	127	177	12.1	600
	B3	136	191	12.9	655	B2L	135	170	10.9	533
	B2	140	198	14.6	748	B1	139	187	11.5	583
Average.....		*137*	*208*	*12.4*	*641*		*134*	*178*	*11.5*	*572*
150–175	C1	160	224	10.5	567	E1	154	173	11.7	576
	C4	162	247	8.7	482	C3	161	191	9.7	493
	E3	163	285	9.7	563	D1	162	187	11.2	566
	E4	164	267	9.8	554	C2	164	184	10.2	514
	C2L	165	227	11.7	629	E5	165	192	11.7	596
	E2	166	251	9.9	552	D4	171	203	9.9	515
	D2	168	259	11.2	633	B1	174	212	11.3	592
	D3	171	269	13.2	749					
	B2	175	238	11.9	651					
Average.....		*166*	*252*	*10.7*	*598*		*164*	*192*	*10.8*	*550*
Over 175	B3	177	217	9.4	495	B2L	176	187	12.6	636
	B4	188	277	8.9	509					
Average.....		*182*	*247*	*9.1*	*502*					

ship among the various age-groups of male and female rats; in fact, the average for females, 11.74 ± 0.20 calories, is higher than that for the males, 11.26 ± 0.24 calories.

It is interesting to note the great difference between the two sexes relative to the variability in the basal metabolism determinations. Expressed per square meter of body surface, the daily metabolism of the males possessed an average deviation for the 23 determinations of 71 calories, and a coefficient of variation (i.e., the standard deviation in per cent of the mean) of 13.5 per cent. On the same basis, the daily metabolism of the females (18 determinations), possessed an average deviation of only 37 calories, and a coefficient of variation of only 8.0 per cent. Expressed per 100 grams body weight, the daily metabolism of the males possessed an average deviation of 1.46 calories and a coefficient of variation of 15.2 per cent. For the females these values are, respectively, 0.87 calorie and 11.0 per cent.

The coefficients of variation for these basal metabolism determinations on rats may be compared with those computed by Harris and Benedict for humans (16). For men, the coefficients found were 9.36 for the basal heat production referred to body weight, and 8.05 for the basal heat production referred to body surface. For women, the coefficients were, respectively, 14.14 and 9.17.

It is also interesting to observe that for both males and females the coefficient of variation of the daily basal metabolism is smaller when expressed per square meter of body surface than when expressed per 100 grams body weight. For the males, the coefficients are 13.5 on the surface basis and 15.2 on the weight basis. For the females, the coefficients are 8.0 on the surface basis and 11.0 on the weight basis. Evidently these determinations of basal metabolism are better equalized for differences in the size of the rats by referring them to surface rather than weight.

This relation seems a legitimate argument for the surface area law. Harris and Benedict (16, p. 147) observed the same relation between the variability of their basal determinations referred to weight and to surface. However, they hesitate to ascribe to this relation any significance as a criterion of the relative merits of the two methods of expressing the intensity of basal metabolism. They point out that, from mathematical considerations only, one would expect the surface area of animals of a given species to possess a smaller percentage variability than the body weight. Hence, they argue, the determined basal heat production divided by the body surface will give a less variable series of ratios than will the basal heat production divided by the body weight. The relation becomes thus a "mathematical necessity," with no physiological significance.

The argument of Harris and Benedict fails to consider one important fact, namely, that the variability of the *ratios* of heat production to either

weight or surface depends not only upon the variability of the original heat production determinations and of the body surfaces and body weights, but also upon the correlations existing between the heat production and the weight and between the heat production and the surface. In fact, since basal heat production is so largely determined by size of body, the degree of correlation is the determining factor in the

TABLE 3

The basal metabolism of rats 30 to 40 days of age

RAT NUMBER	AGE	BODY WEIGHT	BODY SURFACE	LENGTH OF FAST	RESULTS OF RESPIRATION EXPERIMENT					
								Heat produced per day		
					Length	R.Q.	O_2 per day	Total	Per 100 grams body weight	Per square meter body surface
Females										
	days	*gms.*	*sq. cm.*	*hrs.*	*min.*		*liters*	*cals.*	*cals.*	*cals.*
4	32	52	162	19	24	0.71	3.07	14.4	27.7	888
					24	0.71	2.94	13.8	26.5	857
3	39	67	187	19	24	0.71	2.79	13.1	19.5	700
7	36	63	180	18	24	0.73	2.62	12.4	19.7	687
8	38	70	193	34	24	0.71*	2.91	13.6	19.5	707
					24	0.71*	3.34	15.7	22.4	811
6	39	69	191	24	24	0.71*	2.83	13.3	19.2	695
Average.....	*36*	*63*	*181*	*24*			*2.93*	*13.76*	*22.1*	*764*
Males										
2	38	64	182	17	24	0.71	2.40	11.3	17.4	618
					24	0.71	2.18	10.2	16.0	562
1	39	68	189	17	24	0.71	3.43	16.1	23.7	851
					24	0.71	3.25	15.2	22.4	806
5	40	74	200	24	24	0.71*	3.12	14.6	19.8	731
					24	0.71*	3.16	14.8	20.0	741
Average.....	*39*	*69*	*190*	*19*			*2.92*	*13.70*	*19.9*	*718*

* An average respiratory quotient of 0.713 was assumed in these experiments.

matter. Therefore, the smaller variability of basal heat production per square meter of body surface may be fairly interpreted to mean a greater correlation between the two than between basal heat production and the body weight. This greater correlation, may be taken to mean a closer causal relation, certainly of physiological significance.

TABLE 4

The effect of the ingestion of excess amounts of vitamin B on the basal metabolism of rats

VITAMIN SUPPLEMENT DAILY	MALES					FEMALES				
				Heat produced daily					Heat produced daily	
	Rat	Age	Weight	Per 100 grams body weight	Per square meter	Rat	Age	Weight	Per 100 grams body weight	Per square meter
mgm.		*days*	*grams*	*cals.*	*cals.*		*days*	*grams*	*cals.*	*cals.*
10						E1	107	150	11.9	560
						E1	154	173	11.7	576
Average.....							*130*	*161*	*11.8*	*568*
20	C1	123	202	12.9	667	B1	59	84	15.6	609
	C1	160	224	10.5	567	B1	139	187	11.5	583
	E2	108	217	12.1	643	B1	174	212	11.3	592
	E2	166	251	9.9	552	D1	120	172	12.3	605
						D1	162	187	11.2	566
Average.....		*139*	*223*	*11.3*	*605*		*131*	*168*	*12.4*	*591*
40	B2	140	198	14.6	748					
	B2	175	238	11.9	651					
	C2L	118	196	13.2	676	A2	96	128	12.5	556
	C2L	165	227	11.7	629	B2L	135	170	10.9	533
	D2	121	223	12.0	641	B2L	176	187	12.6	636
	D2	168	259	11.2	633	C2	122	166	11.6	527
	E3	114	232	11.7	631	C2	164	184	10.2	514
	E3	163	285	9.7	563					
Average.....		*145*	*232*	*12.0*	*646*		*139*	*167*	*11.6*	*553*
80	A3	93	150	11.5	540	C3	111	171	11.3	551
	B3	136	191	12.9	655	C3	161	191	9.7	493
	B3	177	217	9.4	495					
	D3	171	269	13.2	749					
	E4	113	240	8.1	444					
	E4	164	267	9.8	554					
Average.....		*142*	*222*	*10.8*	*573*		*136*	*181*	*10.5*	*522*
120	A4	91	151	11.9	559	D4	127	177	12.1	600
	B4	134	234	9.6	521	D4	171	203	9.9	515
	B4	188	277	8.9	509					
	C4	110	199	13.6	698					
	C4	162	247	8.7	482					
Average.....		*137*	*222*	*10.5*	*554*		*149*	*190*	*11.0*	*557*
160						E5	115	188	13.4	676
						E5	165	192	11.7	596
Average.....							*140*	*190*	*12.5*	*636*

No distinct effect of age, insofar as this factor varied among the different rats, is evident from the figures of table 2. For successive age groups the basal heat production per day per square meter of body surface for the males was 549, 629, 640, 598 and 502 calories. These results taken at their face value might indicate an increasing basal metabolism up to an age of 125 to 150 days followed by a decrease. The results for the females, however, do not show such a relation, the average basal metabolism for increasing age groups being 582, 584, 572 and 550 calories per square meter of body surface per day. Since investigations on other animals have not indicated that the effect of age on basal metabolism is essentially different for the two sexes, the progressive variation shown by the successive group averages for the male rats should not be considered significant until confirmed by future work.

Although within the age limits of the rats used in this investigation the basal heat production, when referred to body surface, does not seem to vary in intensity with age, it would be expected from investigations on other animals that for younger rats a more intense basal metabolism exists. The experimental results tabulated in table 3, obtained in connection with another investigation by the use of the same methods as those described above, show the correctness of this supposition. These rats ranged in age from 32 to 40 days. Their basal metabolism averaged considerably higher than the older rats considered in table 2, and with few exceptions the individual values in table 3 were also higher than the large majority of the individual values of table 2. These young rats showed an average basal heat production of about 740 calories per square meter of body surface per day. The slight average difference noted between the male and female rats is certainly insignificant in view of the variability existing among individual determinations.

In table 4 the essential data for the individual rats are grouped according to sex and according to the daily dosage of vitamin B supplement. For the groups of male rats receiving respectively 20, 40, 80 and 120 mgm. of vitamin B supplement daily the average basal metabolism was, in the same order, 605, 646, 573 and 554 calories per square meter of body surface per day. For the groups of females receiving increasing amounts of vitamin supplement from 10 mgm. to 160 mgm. daily the average daily basal metabolism was, in order, 568, 591, 553, 522, 557 and 636 calories per square meter of body surface daily. It does not appear from these figures that the varying amounts of vitamin B supplement consumed by the rats exerted any appreciable effect upon their basal metabolism.

SUMMARY AND CONCLUSIONS

For rats ranging in age from 90 to 190 days, the basal heat production of males per square meter of body surface per day appears to be higher

than that for females. The averages found in this investigation were 600 ± 11 calories daily per square meter of body surface for males and 571 ± 7 calories daily per square meter of body surface for females. However, no clear distinction between the sexes is evident in the intensity of the basal metabolism per unit of weight. In fact, the average value obtained for male rats was less than that for female rats.

The basal metabolism of male rats per unit of body surface or of body weight is more variable than that of female rats. In both sex groups, the variability of the basal metabolism referred to body surface was less than that referred to body weight.

Within the age interval above noted, age does not appear to have an appreciable effect in modifying the basal heat production of rats. However, the basal heat production of rats from 30 to 40 days in age is distinctly higher per square meter of body surface, as well as per 100 grams of body weight, than the basal heat production of more mature rats.

The ingestion of excessive amounts of vitamin B up to about 10 times their estimated requirements does not appreciably modify the basal heat production of rats. Therefore, insofar as the basal heat production is a measure of "vitality," it may be concluded that the ingestion of amounts of vitamin B in excess of the minimum requirements for growth and continued well-being does not benefit an animal.

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THE COMPOSITION OF THE GAINS IN WEIGHT AND THE UTILIZATION OF FOOD ENERGY IN GROWING RATS

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Received for publication January 11, 1926

The gain in weight of an animal during its growing period is commonly taken as a measure of the nutritive effect of the food consumed. In the many feeding experiments on rats reported in the literature concerned with the evaluation of different foods and food products as sources of protein and vitamins, the relative rates of growth secured on the different experimental rations used have been taken as satisfactory measures of their relative growth-promoting values. The conception is expressed even more definitely in methods that have been proposed for the quantitative determination of the relative growth-promoting values of different foods as sources of indispensable nutrients. Methods of measuring the protein value of different rations by the increase in weight per gram of protein consumed, as well as methods concerned with the measuring of the vitamin value of food products by determining the minimum intake requisite for a definite rate of increase in weight of experimental animals, assume that the increase in weight of an animal is a satisfactory measure of the nutritive effect of its food.

The assumption that a definite increase in weight of an animal during growth, is a measure of the nutritive effect of the total food consumed is subject to two sources of error, even though the age, the initial weight, and the time interval are definitely prescribed. In the first place the growth secured is not the only nutritive effect of the food consumed, so that the amount of food consumed per unit gain in weight in a definite time depends upon the total amount of food consumed, since the larger this amount, other conditions being equal, the smaller the proportion of it that will be used in functions other than growth, such as maintenance and muscular activity. In the second place, the gain in weight of an animal induced by the consumption of a definite amount of food will vary in composition, particularly in its content of water, fat and energy. Evidently equal gains induced by the consumption of different rations in equal amounts does not mean that the rations possess equal value in any or all respects, if the gain in one case is more "dilute" than the gain in the other.

In view of the rather general acceptance of the assumption in rat feeding experiments that the rate of gain in weight, particularly under certain prescribed conditions, is a satisfactory measure of the nutritive effect of a ration, it was considered profitable in connection with the experiment described in the preceding paper actually to determine the composition of the gains in weight put on by the experimental animals. When each litter was put on experimental feeding, one or more of the individual rats in the litter were killed and analyzed in the routine fashion, except that the gross energy of each carcass was determined directly with the bomb calorimeter. The composition of these check rats was taken as a determination of the composition of the other rats at the time they were put

TABLE 1

Composition of check rats on the basis of the empty weight

RAT	SEX	LIVE WEIGHT	AGE	EMPTY WEIGHT	PERCENTAGE COMPOSITION OF CARCASS MINUS CONTENTS OF THE INTESTINAL TRACT				
					Dry matter	Ether extract	Ash	Total nitrogen	Energy in cal. calories per gram
		grams	*days*	*grams*					
A5	f	81	41	78*	29.77	10.09	3.03	2.56	1856
B6	m	68	32	65*	26.08	5.78	3.35	2.75	1364
B7	m	75	32	72*	26.60	6.61	3.31	2.57	1433
B8	f	76	32	73*	26.15	6.71	3.30	2.70	1478
B9	f	65	32	62*	28.07	6.45	3.44	2.92	1480
C5	f	62	32	59	27.36	4.73	3.68	2.80	1396
C6	f	58	32	55	25.75	4.36	3.24	2.69	1409
C7	m	58	32	54	28.92	4.30	3.91	3.06	1479
D5	m	70	30	66	27.07	5.94	3.07	2.62	1504
E6	m	64	32	62	27.43	7.32	3.25	2.58	1650
Average.....		68	33	65	27.32	6.23	3.36	2.72	1505

* The weight of the contents of the intestinal tract was not taken in these cases, but was assumed to equal 3.0 grams, the average weight for the last five rats.

upon experimental feeding. When the litters were taken off the experiment, the individual rats were immediately killed and analyzed in the same way as the check rats. A comparison of the composition of these rats with their estimated composition at the beginning of the feeding period permits an estimation of the composition of the tissues produced during growth. In all cases, the contents of the gastro-intestinal tract were removed from the carcasses before analysis.

EXPERIMENTAL RESULTS. The composition of the check rats killed at ages ranging from 30 to 41 days is shown in table 1. The individual analyses agree fairly well among themselves except for the values relating to ether extract and gross energy. There seems to be a rather distinct

difference in ether-extract content of the rats from different litters. For example, the rats of litter C seem to possess a distinctly lower content of ether extract than the rats of litter B. The one rat killed in each of litters A and E showed a higher fat content than the members of the other three litters. In view of this fact, it would be advisable to use the average

TABLE 2

Composition of test rats on the basis of the empty weight

RAT	SEX	LIVE WEIGHT	AGE	EMPTY WEIGHT	PERCENTAGE COMPOSITION OF CARCASS MINUS CONTENTS OF THE INTESTINAL TRACT				
					Dry matter	Ether extract	Ash	Total nitrogen	Energy in sm. calories per gram
		grams	*days*	*grams*					
A1	m	109	97	106	23.42	3.30	4.06	2.97	1228
A2	f	123	97	119	30.45	8.49	4.45	3.27	1867
A3	m	154	97	150	31.29	9.11	3.69	3.38	1901
A4	m	158	97	155	31.36	11.13	4.21	3.24	2000
B1	f	228	188	222	35.41	11.38	3.56	3.00	2124
B2	m	241	188	238	39.24	14.09	3.78	3.15	2516
B2L	f	185	188	183	41.48	16.90	4.54	2.98	2647
B3	m	223	188	220	40.20	15.40	4.46	3.09	2647
B4	m	280	188	278	36.58	12.18	3.69	3.10	2179
C1	m	240	165	225	40.28	17.46	3.74	3.21	2567
C2	f	192	165	185	41.11	17.85	3.82	2.94	2446
C2L	m	225	165	221	31.88	9.28	4.05	2.80	1603
C3	f	202	165	196	42.76	21.27	6.21	2.54	2672
C4	m	266	165	256	36.74	12.70	3.68	3.06	2230
D1	f	186	173	183	37.77	13.78	4.55	3.01	2367
D2	m	264	173	260	33.80	15.21	3.25	2.41	2294
D3	m	267	173	263	39.07	15.86	4.06	3.08	2652
D4	f	205	173	200	44.30	24.46	3.07	2.40	3000
E1	f	192	169	184	37.43	15.13	3.36	3.02	2290
E2	m	264	169	253	42.10	20.43	3.05	3.12	2738
E3	m	290	169	280	42.95	23.48	3.17	2.80	3043
E4	m	272	169	266	41.28	19.64	3.87	3.03	2639
E5	f	193	169	188	45.12	22.98	3.02	2.57	3140

composition of the check rats in each litter in estimating the composition of the gains in weight of the other rats in the same litter at the end of their period of experimental feeding, provided several check rats had been analyzed from each litter. Unfortunately, in each of litters A, D and E, only one animal is represented among the control rats. Therefore, in using the analyses of the check rats in the estimation of the composition

of the gains in weight of the other rats, the average analysis of the ten rats was used for all litters.

The percentage composition of the rats analyzed at the end of the experimental feeding periods is given in table 2, while the weights of the different chemical constituents and the total gross energy in the empty carcasses are tabulated in table 3. From these weights and from the

TABLE 3

Weights of nutrients and calories of energy in the empty carcass of the test rats

RAT	SEX	AGE	EMPTY WEIGHT	DRY MATTER	ETHER EXTRACT	ASH	NITROGEN	GROSS ENERGY
		days	*grams*	*grams*	*grams*	*grams*	*grams*	*calories*
A1	m	97	106	24.83	3.50	4.30	3.15	130
A2	f	97	119	36.36	10.14	5.32	3.90	223
A3	m	97	150	46.94	13.67	5.53	5.07	285
A4	m	97	155	48.67	17.27	6.53	5.03	310
B1	f	188	222	78.57	25.26	7.91	6.66	471
B2	m	188	238	93.31	33.50	9.00	7.48	598
B2L	f	188	183	75.91	30.93	8.31	5.45	484
B3	m	188	220	88.29	33.81	9.79	6.78	581
B4	m	188	278	101.62	33.84	10.24	8.61	605
C1	m	165	225	90.84	39.38	8.43	7.24	579
C2	f	165	185	76.17	33.07	7.08	5.44	453
C2L	m	165	221	70.58	20.55	8.96	6.21	355
C3	f	165	196	83.63	41.61	12.14	4.97	523
C4	m	165	256	94.12	32.54	9.42	7.83	571
D1	f	173	183	69.23	25.26	8.34	5.52	434
D2	m	173	260	87.85	39.53	8.44	6.27	596
D3	m	173	263	102.75	41.71	10.67	8.09	697
D4	f	173	200	88.69	48.97	6.14	4.81	601
E1	f	169	184	68.79	27.81	6.18	5.55	421
E2	m	169	253	106.67	51.78	7.72	7.91	694
E3	m	169	280	120.27	65.73	8.88	7.85	852
E4	m	169	266	109.64	52.16	10.28	8.06	701
E5	f	169	188	84.91	43.24	5.69	4.84	591

estimated composition of the rat at the beginning of the experiment, using the average composition of the check rats for this purpose, the composition of the gains in weight was estimated, as shown in table 4. The estimated composition of the gains in weight is summarized in table 5 in grams and in table 6 in per cent.

From a study of table 6, it appears that the gains in tissue put on by growing rats vary widely in composition, even though the ration[1] con-

[1] For the composition of the rations used in this feeding test, see page 386.

TABLE 4

The estimated composition of the gains in weight of the test rats

RAT		EMPTY WEIGHT	DRY MATTER	ETHER EXTRACT	ASH	TOTAL NITROGEN	GROSS ENERGY
		grams	*grams*	*grams*	*grams*	*grams*	*calories*
A1	Final	106	24.8	3.5	4.30	3.15	130
	Initial	81	22.1	5.0	2.72	2.20	122
	Gain, grams	25	2.7	−1.5	1.58	0.95	8
	Gain, per cent*		10.8		6.32	3.80	0.32
A2	Final	119	36.4	10.1	5.32	3.90	223
	Initial	81	22.1	5.0	2.72	2.20	122
	Gain, grams	38	14.3	5.1	2.60	1.70	101
	Gain, per cent*		37.6	13.4	6.84	4.47	2.66
A3	Final	150	46.9	13.7	5.53	5.07	285
	Initial	86	23.5	5.4	2.89	2.34	129
	Gain, grams	64	23.4	8.3	2.64	2.73	156
	Gain, per cent*		36.6	13.0	4.12	4.27	2.44
A4	Final	155	48.7	17.3	6.53	5.03	310
	Initial	82	22.4	5.1	2.76	2.23	123
	Gain, grams	73	26.3	12.2	3.77	2.80	187
	Gain, per cent*		36.0	16.7	5.16	3.84	2.56
B1	Final	222	78.6	25.3	7.91	6.66	471
	Initial	60	16.4	3.7	2.02	1.63	90
	Gain, grams	162	62.2	21.6	5.89	5.03	381
	Gain, per cent*		38.4	13.3	3.64	3.10	2.35
B2	Final	238	93.3	33.5	9.00	7.48	598
	Initial	58	15.8	3.6	1.95	1.58	87
	Gain, grams	180	77.5	9.9	7.05	5.90	511
	Gain, per cent*		43.0	16.6	3.92	3.28	2.84
B2L	Final	183	75.9	30.9	8.31	5.45	484
	Initial	64	17.5	4.0	2.15	1.74	96
	Gain, grams	119	58.4	26.9	6.16	3.71	388
	Gain, per cent*		49.0	22.6	5.18	3.12	3.26
B3	Final	220	88.3	33.8	9.79	6.78	581
	Initial	63	17.2	3.9	2.12	1.71	95
	Gain, grams	157	71.1	29.9	7.67	5.07	486
	Gain, per cent*		45.3	19.0	4.89	3.23	3.10
B4	Final	278	101.6	33.8	10.24	8.61	605
	Initial	63	17.2	3.9	2.12	1.71	95
	Gain, grams	215	84.4	29.9	8.12	6.90	510
	Gain, per cent*		39.3	13.9	3.78	3.21	2.37

TABLE 4—*Continued*

RAT		EMPTY WEIGHT	DRY MATTER	ETHER EXTRACT	ASH	TOTAL NITROGEN	GROSS ENERGY
		grams	*grams*	*grams*	*grams*	*grams*	*calories*
C1	Final	225	90.8	39.4	8.43	7.24	579
	Initial	69	18.9	4.3	2.32	1.88	104
	Gain, grams	156	71.9	35.1	6.11	5.36	475
	Gain, per cent*		46.1	22.5	3.92	3.44	3.04
C2	Final	185	76.2	33.1	7.08	5.44	453
	Initial	67	18.3	4.2	2.25	1.82	101
	Gain, grams	118	57.9	28.9	4.83	3.62	352
	Gain, per cent*		49.1	24.5	4.09	3.07	2.98
C2L	Final	221	70.6	20.5	8.96	6.21	355
	Initial	63	17.2	3.9	2.12	1.71	95
	Gain, grams	158	53.4	16.6	6.84	4.50	260
	Gain, per cent*		33.8	10.5	4.33	2.85	1.65
C3	Final	196	83.6	41.6	12.14	4.97	523
	Initial	63	17.2	3.9	2.12	1.71	95
	Gain, grams	133	66.4	37.7	10.02	3.26	428
	Gain, per cent*		49.9	28.3	7.53	2.45	3.22
C4	Final	256	94.1	32.5	9.42	7.83	571
	Initial	63	17.2	3.9	2.12	1.71	95
	Gain, grams	193	76.9	28.6	7.30	6.12	476
	Gain, per cent*		39.8	14.8	3.78	3.17	2.47
D1	Final	183	69.2	25.3	8.34	5.52	434
	Initial	64	17.5	4.0	2.15	1.74	96
	Gain, grams	119	51.7	21.3	6.19	3.78	338
	Gain, per cent*		43.4	17.9	5.20	3.18	2.84
D2	Final	260	87.8	39.5	8.44	6.27	596
	Initial	61	16.7	3.8	2.05	1.66	92
	Gain, grams	199	71.1	35.7	6.39	4.61	504
	Gain, per cent*		35.7	17.9	3.21	2.32	2.53
D3	Final	263	102.7	41.7	10.67	8.09	697
	Initial	68	18.6	4.2	2.28	1.85	102
	Gain, grams	195	84.1	37.5	8.39	6.24	595
	Gain, per cent*		43.1	19.2	4.21	3.20	3.05
D4	Final	200	88.7	49.0	6.14	4.81	601
	Initial	64	17.5	4.0	2.15	1.74	96
	Gain, grams	136	71.2	45.0	3.99	3.07	505
	Gain, per cent*		52.4	33.1	2.93	2.26	3.71

TABLE 4—*Concluded*

RAT		EMPTY WEIGHT	DRY MATTER	ETHER EXTRACT	ASH	TOTAL NITROGEN	GROSS ENERGY
		grams	*grams*	*grams*	*grams*	*grams*	*calories*
E1	Final	184	68.8	27.8	6.18	5.55	421
	Initial	64	17.5	4.0	2.15	1.74	96
	Gain, grams	120	51.3	23.8	4.03	3.81	325
	Gain, per cent*		42.7	19.8	3.36	3.17	2.71
E2	Final	253	106.7	51.8	7.72	7.91	694
	Initial	67	18.3	4.2	2.25	1.82	101
	Gain, grams	186	88.4	47.6	5.47	6.09	593
	Gain, per cent*		47.5	25.6	2.94	3.27	3.19
E3	Final	280	120.3	65.7	8.88	7.85	852
	Initial	67	18.3	4.2	2.25	1.82	101
	Gain, grams	213	102.0	61.5	6.63	6.03	751
	Gain, per cent*		47.9	28.9	3.11	2.83	3.53
E4	Final	266	109.6	52.2	10.28	8.06	701
	Initial	69	18.9	4.3	2.32	1.88	104
	Gain, grams	197	90.7	47.9	7.96	6.18	597
	Gain, per cent*		46.0	24.3	4.04	3.14	3.03
E5	Final	188	84.9	43.2	5.69	4.84	591
	Initial	60	16.4	3.7	2.02	1.63	90
	Gain, grams	128	68.5	39.5	3.67	3.21	501
	Gain, per cent*		53.5	30.9	2.87	2.51	3.91

* Percentage composition and energy value per gram of estimated gains in empty weight.

sumed is the same. It is particularly noteworthy that the fat content of the added tissue may vary widely, even attaining a value as high as 33 per cent. Corresponding variations in the energy content per gram of gain and in the percentage dry matter are revealed by these analyses. No clear sex differences in the composition of gains are evident, although for each litter of rats except litter A the highest percentage of ether extract and the highest quantity of energy per gram of gain was exhibited by one of the two females in the litter.

It is remarkable that rats on the same ration and gaining approximately the same in weight in the same interval of time may nevertheless put on gains differing greatly in composition. A comparison of rats C1 and C2L is an illustration of this point. These two rats gained approximately the same in empty weight, namely, 156 and 158 grams, in the same time interval on practically the same amounts (see table 7) of the same ration. However, the gain in the first case possessed a dry matter content of 46.1 per cent and in the second case a dry matter content of 33.8 per cent. The

fat content of the gain of rat C1 was 22.5 per cent and of rat C2L, 10.5 per cent. The energy value per gram of gain was 3.04 cals. in the first case and 1.65 cals. in the second case. Similar discrepancies may be found within each litter of rats; for example, rats B1 and B3, D2 and D3,

TABLE 5

Estimated quantities of chemical constituents and of gross energy in the gains in weight

RAT	SEX	LENGTH OF EXPERIMENT	FINAL LIVE WEIGHT*	TOTAL GAIN IN EMPTY WEIGHT	COMPOSITION OF GAIN				
					Dry matter	Ether extract	Ash	Total nitrogen	Gross energy
		days	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*calories*
A1	m	56	118	25	2.7	−1.5	1.58	0.95	8
A2	f	56	134	38	14.3	5.1	2.60	1.70	101
A3	m	56	165	64	23.4	8.3	2.64	2.73	156
A4	m	56	169	73	26.3	12.2	3.77	2.80	187
B1	f	156	228	162	62.2	21.6	5.89	5.03	381
B2	m	156	241	180	77.5	29.9	7.05	5.90	511
B2L	f	156	185	119	58.4	26.9	6.16	3.71	388
B3	m	156	223	157	71.1	29.9	7.67	5.07	486
B4	m	156	280	215	84.4	29.9	8.12	6.90	510
C1	m	133	240	156	71.9	35.1	6.11	5.36	475
C2	f	133	192	118	57.9	28.9	4.83	3.62	352
C2L	m	133	225	158	53.4	16.6	6.84	4.50	260
C3	f	133	202	133	66.4	37.7	10.02	3.26	428
C4	m	133	266	193	76.9	28.6	7.30	6.12	476
D1	f	143	186	119	51.7	21.3	6.19	3.78	338
D2	m	143	264	199	71.1	35.7	6.39	4.61	504
D3	m	143	267	195	84.1	37.5	8.39	6.24	595
D4	f	143	205	136	71.2	45.0	3.99	3.07	505
E1	f	137	192	120	51.3	23.8	4.03	3.81	325
E2	m	137	264	186	88.4	47.6	5.47	6.09	593
E3	m	137	290	213	102.0	61.5	6.63	6.03	751
E4	m	137	272	197	90.7	47.9	7.96	6.18	597
E5	f	137	193	128	68.5	39.5	3.67	3.21	501

* The rats in litter A were slaughtered two days after the feeding period was terminated. The final weights in this table, therefore, are different from the slaughter weights given in preceding tables. All other litters were killed on the last day of the feeding period.

and E1 and E5. It appears that a gain in weight of the live rat, even for a definite age, from a definite initial weight, and during a feeding period of definite length, is not a definite measure of the nutritive effect of the food. Differences in the intake of food do not appear to be responsible for these differences in the composition of gains.

The ration consumed by the rats analyzed in this experiment (see page 386) was found by direct determination to contain 4.26 cals. of gross energy per gram. Since the total food intake of all the rats was measured, it is possible to compute the total intake of energy as the basis of computations concerning the utilization of energy during growth. From the

TABLE 6

Estimated percentage composition and energy value per gram of the gains in empty weight

RAT	SEX	LENGTH OF EXPERIMENT	FINAL LIVE WEIGHT	GAIN IN EMPTY WEIGHT	COMPOSITION OF GAINS				
					Dry matter	Ether extract	Ash	Total nitrogen	Gross energy per gram
		days	*grams*	*grams*	*per cent*	*per cent*	*per cent*	*per cent*	*calories*
A1	m	56	118	25	10.8		6.32	3.80	0.32
A2	f	56	134	38	37.6	13.4	6.84	4.47	2.66
A3	m	56	165	64	36.6	13.0	4.12	4.27	2.44
A4	m	56	169	73	36.0	16.7	5.16	3.84	2.56
B1	f	156	228	162	38.4	13.3	3.64	3.10	2.35
B2	m	156	241	180	43.0	16.6	3.92	3.28	2.84
B2L	f	156	185	119	49.0	22.6	5.18	3.12	3.26
B3	m	156	223	157	45.3	19.0	4.89	3.23	3.10
B4	m	156	280	215	39.3	13.9	3.78	3.21	2.37
C1	m	133	240	156	46.1	22.5	3.92	3.44	3.04
C2	f	133	192	118	49.1	24.5	4.09	3.07	2.98
C2L	m	133	225	158	33.8	10.5	4.33	2.85	1.65
C3	f	133	202	133	49.9	28.3	7.53	2.45	3.22
C4	m	133	266	193	39.8	14.8	3.78	3.17	2.47
D1	f	143	186	119	43.4	17.9	5.20	3.18	2.84
D2	m	143	264	199	35.7	17.9	3.21	2.32	2.53
D3	m	143	267	195	43.1	19.2	4.21	3.20	3.05
D4	f	143	205	136	52.4	33.1	2.93	2.26	3.71
E1	f	137	192	120	42.7	19.8	3.36	3.17	2.71
E2	m	137	264	186	47.5	25.6	2.94	3.27	3.19
E3	m	137	290	213	47.9	28.9	3.11	2.83	3.53
E4	m	137	272	197	46.0	24.3	4.04	3.14	3.03
E5	f	137	193	128	53.5	30.9	2.87	2.51	3.91

results of another experiment, which will be described later, it may be estimated that approximately 15 per cent of the gross energy of the ration in this experiment was excreted in the feces and urine, only 85 per cent being metabolizable in the sense that Armsby has given to this term. It may be computed, therefore, that each gram of the ration contained 3.62 cals. (85 per cent of 4.26) of metabolizable energy.

The purpose of table 7 is to obtain a percentage expressing that fraction of the metabolizable energy intake of each rat which can be accounted for in its basal heat production and in the energy contained in the tissues added to its body during growth. The basal heat production was com-

TABLE 7

The utilization of energy by growing rats

RAT NUMBER	SEX	INITIAL WEIGHT	FINAL WEIGHT	AVERAGE WEIGHT	LENGTH OF EXPERIMENT	TOTAL GAIN	TOTAL FOOD CONSUMED	TOTAL INTAKE OF METABOLIZABLE ENERGY	AVERAGE BODY SURFACE	AVERAGE BASAL HEAT PRODUCTION PER DAY	TOTAL BASAL HEAT PRODUCTION	ENERGY ADDED TO BODY	TOTAL FOOD ENERGY ACCOUNTED FOR	UTILIZATION OF METABOLIZABLE ENERGY IN MAINTENANCE AND GROWTH
		grams	*grams*	*grams*	*days*	*grams*	*grams*	*calories*	*sq. cm.*	*calories*	*calories*	*calories*	*calories*	*per cent*
A1	m	84	118	96	56	34	360	1302	238	14.28	800	8	808	62
A2	f	84	134	106	56	50	407	1473	255	14.56	815	101	916	62
A3	m	89	165	124	56	76	512	1852	282	16.92	948	156	1104	60
A4	m	85	169	130	56	84	533	1928	291	17.46	978	187	1165	60
B1	f	63	228	159	156	165	1455	5264	333	19.01	2966	381	3347	63
B2	m	61	241	173	156	180	1582	5724	353	21.18	3304	511	3815	67
B2L	f	67	185	146	156	118	1426	5159	314	17.93	2797	388	3185	62
B3	m	66	223	167	156	157	1552	5615	345	20.70	3229	486	3715	66
B4	m	66	280	201	156	214	1717	6212	390	23.40	3650	510	4160	67
C1	m	72	240	170	133	168	1391	5033	350	21.00	3276	475	3751	75
C2	f	70	192	150	133	122	1283	4642	320	18.27	2850	352	3202	69
C2L	m	66	225	169	133	159	1374	4971	348	20.88	3257	260	3517	71
C3	f	66	202	157	133	136	1307	4729	331	18.90	2948	428	3376	71
C4	m	66	266	181	133	200	1457	5271	364	21.84	3407	476	3883	74
D1	f	67	186	154	143	119	1440	5210	326	18.61	2903	338	3241	62
D2	m	64	264	193	143	200	1617	5850	380	22.80	3556	504	4060	69
D3	m	71	267	199	143	196	1687	6104	387	23.22	3622	595	4217	69
D4	f	67	205	157	143	138	1475	5337	331	18.90	2948	505	3453	65
E1	f	67	192	144	137	125	1216	4399	311	17.76	2771	325	3096	70
E2	m	70	264	195	137	194	1440	5210	382	22.92	3576	593	4169	80
E3	m	70	290	210	137	220	1531	5539	402	24.12	3763	751	4514	81
E4	m	72	272	207	137	200	1550	5608	398	23.88	3725	597	4322	77
E5	f	63	193	161	137	130	1351	4888	337	19.24	3001	501	3502	72

puted from the estimated body surface of the rat, using the Meeh formula with a constant of 11.36 (see page 380), and from the average values for the basal heat production of male and female rats obtained in the preceding investigation, namely, 600 cals. per square meter of body surface

per day for male rats and 571 cals. per square meter of body surface per day for female rats. The daily figure obtained in this way for each rat, depending upon its sex, multiplied by the number of days in the feeding test, gives the total basal heat production of the rat for the entire growth period. The figures thus obtained are added to the corresponding figures for the energy content of the gains secured, given in table 5, and the sum is divided by the intake of metabolizable energy to get a percentage utilization. These percentages will be found in the last column to the right of table 7. While marked differences appear between percentages obtained for different litters, the results within any one litter generally agree fairly well. In general 60 to 75 per cent of the metabolizable energy consumed by these rats could be accounted for by their basal heat production and by the energy recovered in the tissue formed during growth. Litter E showed an efficiency slightly higher than these figures.

It is somewhat surprising to find that such a small percentage of the metabolizable energy consumed by rats is represented in the gains in weight put on during growth. In all but the last litter, the energy stored in the body constituted only from 6 to 10 per cent of the metabolizable energy consumed. In litter E, three of the rats gave a value of 11 per cent and one, E3, a value of 14 per cent.

Perhaps the most interesting feature of these results is that for each litter except litter A, which was on experiment for the shortest period of time, the female rats consistently gave the lowest percentages. The size of this percentage may be considered to depend upon two factors, namely, the specific dynamic effect of the food and the muscular activity of the rat. The greater either of these two factors, the smaller will be the resulting percentage. It is a fair assumption that the specific dynamic effect of the food was approximately the same for all rats, regardless of sex. Therefore, the lower percentage utilization of the metabolizable energy consumed obtained with the female rats may be interpreted to mean that the voluntary activity of female rats is greater than the voluntary activity of the male rats. This conclusion confirms the results of Slonaker[2] on the voluntary activity of the albino rat.

The fact that 80 per cent of the metabolizable energy intake of a rat may be accounted for by its basal heat production and the energy content of the tissue added to its body suggests strongly that the specific dynamic effect of food in the rat is very slight. In fact, we have run respiration experiments on rats before and after feeding, by the same method as that already reported (see page 387), with results indicating either no heat effect of food or only a slight effect. The results, however, are too few in number and too variable to be reported finally at the present time.

[2] Slonaker, J. R. Journ. Animal Behavior, 1912, ii, 20.

An experiment carried out with other purposes in view confirms the suspicion that the specific dynamic effect of food is very slight in the rat. In this experment 6 rats varying in weight from 67 to 225 grams were maintained for several months at constant weight on a ration consisting of 85 per cent of ground yellow corn and 15 per cent of tankage, a packing-house by-product. Throughout the maintenance period they were confined in small individual wire cages. Towards the end of the maintenance period each rat was put in a metabolism dish similar to that used in the protein investigations of this laboratory.[3] For a period of seven days the urine and feces of these rats were collected and an energy determination was made upon the composite weekly sample. The results of this test are shown in table 8. In this table will be found the weights of the rats and their body surfaces as estimated by the Meeh formula (see page 380).

TABLE 8

A comparison of the quantity of metabolizable energy required by rats for maintenance and their estimated basal heat production

RAT	WEIGHT	SURFACE	DAILY FOOD	GROSS ENERGY	METABOLIZABLE ENERGY	BASAL HEAT PRODUCTION	PER CENT UTILIZATION
	grams	*sq. cm.*	*grams*	*calories*	*calories*	*calories*	
1	218	411	7.50	30.11	25.64	24.08	93.9
2	265	469	8.70	34.92	28.40	27 48	96.8
3	155	328	6.00	24.09	20.00	19.22	96 1
4	141	307	6.00	24.09	19.71	17.99	91.3
5	146	314	6.00	24.09	19.85	18.40	92.7
6	67	187	3.60	14.45	11.63	10.96	94.2

Note: These data were obtained by Mr. L. A. Luhnow and Miss M. H. Keith.

In the fourth column is the amount of food required for maintenance of body weight over several months of feeding. The gross energy value of this food is given in the next column and in the next column will be found the metabolizable energy intake obtained by deducting from the gross energy intake the energy content of the excreta. The basal heat production has been estimated on the assumption of an average metabolism of 585 cals. per square meter of body surface per day, since the sex of these rats, unfortunately, was not noted in the experimental records. This figure is the average for males and females as determined in a preceding experiment. In the final column of this table the estimated basal heat production per day is expressed as a percentage of the daily intake of metabolizable energy. If it may be assumed that the basal heat production of rats that have been maintained at constant weight over a considerable period of time has not been lowered below that obtained on rats nourished on higher food levels, the results of this table must be taken to

[3] Mitchell, H. H. and G. G. Carman. Journ. Biol. Chem., 1924, lx, 613.

mean that the heating effect of food consumed in quantities sufficient only to maintain constant body weight is very slight and possibly non-existent.

CONCLUSIONS

The composition of the gains in weight made by growing rats varies considerably, even though the ration consumed, the initial weight of the rats, the length of the growth period, and the rate of growth may be the same. It follows that different rates of growth on different methods of feeding may not indicate different nutritive effects of the rations. Therefore, the gain in weight of an animal, no matter how carefully experimental conditions may be prescribed, is not a definite measure of the nutritive effect of the ration fed, but is subject to considerable variation due to the operation of unknown factors.

In growing rats, as much as 60 to 80 per cent of the metabolizable energy consumed in the food may be accounted for by the basal heat production of the rat and by the energy content of the gains in weight produced. In general, smaller percentages are obtained for female than for male rats, indicating reasonably that female rats are more active during their growth period than are male rats. The gains in weight alone account for only a small proportion of the intake of metabolizable energy, namely, from 6 to 14 per cent.

The specific dynamic effect of food in the growing rat is probably small, while in rats on a maintenance ration, it is probably entirely inappreciable.

THE ACTION OF ACIDS ON TURTLE HEART MUSCLE WITH REFERENCE TO THE PENETRATION OF ANIONS

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Received for publication January 13, 1926

The apparent penetration of turtle atrial tissue by bicarbonate can be demonstrated by treating the tissue with $NaHCO_3$-Ringer's solution and subsequently washing and extracting it with $NaHCO_3$-free solution, when the extracted bicarbonate can be titrated with dilute HCl. Though experiments of this kind are no more than qualitative, they show that the tissue absorbs a quantity of bicarbonate practically equivalent to that present in an equal volume of the solution to which the tissue is exposed.

The distribution of bicarbonate might be the result of direct migration of Na^+ and HCO_3^- ion pairs between fluid and cell, or it might be the result of an exchange of anions such as has been demonstrated in the mammalian red blood cell. In respect to the latter, it is generally recognized that the bicarbonate exchange between corpuscle and plasma is effected without exchange of Na^+, K^+ or other cations (Van Slyke, Wu and McLean, 1923); when a migration of HCO_3^- occurs in one direction, a simultaneous and equivalent migration of Cl^- occurs in the opposite direction. This exchange of anions fulfills two theoretically necessary conditions: first, the maintenance of electrical neutrality (equivalent concentrations of cations and anions) in both cell and fluid; and second, the maintenance of the osmotic relations between cell and fluid (since the non-migratory cations restrain equivalent concentrations of anions, a state of impermeability to cations is equivalent osmotically to a state of impermeability to both cations and anions). The former condition is, of course, of universal application, and the latter condition applies to any cell which is a more or less rigid osmotic system; since the majority of tissues, including heart muscle, are such, the direct migration of cation and anion pairs may be considered on this *a priori* basis to be improbable. But so long as the process is viewed as one of migration, whether of cation and anion pairs, or of anions alone, it is to be expected that a condition of permeability to all anions would exist.

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With a view to testing this expectation, an attempt is made in the following experiments to examine by a physiological method the cell-fluid distribution of bicarbonate and to obtain evidence on the possible penetration of other anions. In the light of experiments discrediting the migration between cell and fluid of either cation and anion pairs, or of anions alone, other experiments were performed which bear indirectly on the *modus operandi* of this anion distribution.

The method may be described as one of physiological triangulation. Carbonic acid has a definite and measurable effect on the contractility of heart muscle; by observing the effects of carbonic acid in the presence of various concentrations of bicarbonate ions, it is possible from the mass law to make certain calculations concerning the concentration of bicarbonate ions in the muscle. The validity of the method depends on the supposition (which is strongly supported by the experimental evidence) that the action of CO_2 on the contractility of the muscle is due solely to its acid properties. The extension of the method to other reactants, such as lactates, simultaneously with or independently of bicarbonate, rests simply on the interpretation of the results obtained with the latter.

The earliest account noted of the action of CO_2 on cardiac muscle is that of Klug (1879, quoted by Gaskell, 1880–82) who stated that excessive amounts of carbonic reduced the amplitude of contraction of the frog heart and ultimately brought about diastolic arrest. Gaskell (1880–82) found that a NaCl solution of lactic acid had a similar effect; no recovery was observed when salt solution was substituted for the acid perfusing fluid, but recovery to normal contractility was fairly rapid when the perfusing fluid contained NaOH. Perfusion of a normal heart with alkaline salt solution caused an increase in tone and ultimate arrest in a tonic condition. (The rôle of smooth muscle fibers in the "tonic" contractions of turtle atria was not then recognized, and Gaskell was partly concerned in his experiments with the relation of "systolic" to "tonic" contraction.)

The next observations were those of Jerusalem and Starling (1910) on the mammalian heart-lung preparation. It was found that the administration of a moderate concentration of CO_2 in the respiratory mixture improved the heart action, increasing the relaxation and thereby the volume output. The improvement in volume output was referred by Ketchem, King and Hooker (1912–13) to vascular dilatation in the pulmonary bed on the basis of the vasodilatory action of CO_2, a phenomenon which had been described by Gaskell (1880–82) Severini (1878, quoted by Gaskell), Bayliss (1901) and Hooker (1911, 1912–13). Mines (1910) pointed out that increased alkalinity induces "tonic" contraction in heart muscle and emphasized the fact that in a heart-lung preparation where the blood is alkaline from loss of CO_2, the administration of CO_2 would lead to increased dilatation and increased volume output by simply correcting this abnormal condition. That acids in general and CO_2 in particular reduce the contractility of cardiac muscle is indicated by the observations of Göthlin (1901), Backman (1907), Mines (1910 and 1913b), Straub (1911), Clark (1913–14), Burridge (1913 and 1920–21), and Dale and Thacker (1913–14). Andrus (1919) and Andrus and Carter (1921) have stated that a moderate CO_2 tension, in contrast to a low CO_2 tension, improved the contractility of cardiac muscle.

Mechanical method of recording contractility. The isotonic contraction of atrial muscle was recorded while the muscle was contained in a closed chamber filled with Ringer's solution containing known quantities of CO_2. The muscle chamber was a domed, cylindrical, Pyrex vessel 6.5 cm. × 15.0 cm., holding 370 cc. The dome contained three orifices, 0.5 cm. in diameter, aligned along one diameter of the chamber and 1.7 cm. apart. A thread connecting the muscle to the muscle lever passed through the orifice on one side; a thread supporting an annular glass stirrer within the chamber passed through the opposite orifice; and the central orifice permitted the insertion of a pipette for the removal of samples of solution. A three-holed rubber stopper closed the bottom of the chamber. One hole carried an U-shaped glass tube which was filled with mercury and in one end of which was sealed a platinum wire; the latter served as an anchor for the muscle and as an electrode for stimulation. A glass rod, reaching nearly to the top of the chamber, was inserted in the opposite hole to form a carrier for the glass ring stirrer which was raised and lowered by a thread passing out through the dome. A T-tube was inserted from the outside into the center hole; this tube was connected with aspirating bottles containing the experimental solutions on one side and with a waste drain on the other. With this arrangement the chamber could be filled and emptied without disturbing the muscle; and through the central orifice in the dome, smaller quantities of fluid could be introduced or removed as desired. When working with solutions of appreciable CO_2 tension the chamber was filled so that the fluid rose slightly in all the dome orifices, making an effective seal. The stirrer was mechanically raised and lowered about once a second, often enough to keep the fluid in the chamber circulating steadily but not vigorously.

A silver wire entering the chamber through the central orifice and following the wall nearly to the bottom was used as a second electrode. The stimulating current entered the muscle from the anchoring electrode and passed diffusely into the solution. Pairs of make-and-break induction shocks, obtained from a Harvard coil activated by a time clock and relay, were used for stimulation. The interval between make and break was reduced so that the make shock being effective, the break shock fell within the long refractory period of the muscle.

The muscle, anchored at the bottom to the electrode, was attached by a silk thread to an aluminum heart muscle lever placed above the chamber and arranged to write on a drum carrying smoked paper. The muscle lever carried a fine chain, attached 5 cm. from the axis; the other end of this chain was fastened to a movable slider on a vertical graduated support. By varying the height of the slider the weight of chain bearing on the muscle lever could be easily and reproducibly adjusted. The dimensions of the muscle lever, including the parchment paper writing point, were 5 cm. and 22.5 cm., magnification = 4.5.

The chamber and the muscle lever were separately supported from a heavy base by suitable gear so that fine adjustments of their heights could be made during operation. The muscle lever itself was supported from a double-spring and thumbscrew arrangement that permitted fine adjustments of the axis of rotation in two planes; in one plane perpendicular to the axis of the drum, controlling the pressure of the writing point on the drum surface; and in a second plane tangential to the drum surface, controlling the pressure of the writing point at the top and bottom of its journey.

Chemical methods. The experiments involve principally the observation of the action of CO_2 on the contractility of the muscle when immersed in a Ringer's solution of constant bicarbonate content. The exposure to CO_2 was accomplished by adding to the solution in the chamber definite quantities of this same solution saturated with CO_2. The reverse process of decreasing the CO_2 concentration could be accomplished in part by removing some of the fluid in the chamber and refilling with the alkaline bicarbonate-Ringer's solution. Usually, however, the CO_2 concentration was increased by one or more steps and at the conclusion of a series of observations the solution was replaced entirely by the alkaline bicarbonate solution and a "recovery period" allowed.

The Ringer's solution was made up with accurately known concentrations of $NaHCO_3$. This salt was added from a stock solution which was 0.58 N in respect to total base (hot titration with bromocresol purple) and 0.57 N in respect to CO_2 (Van Slyke analysis). The Ringer's solution was frequently titrated to check the $NaHCO_3$ content.

The other components of the Ringer's solution were added in the proportion in general use (cf. Bayliss, 1924; Mines, 1910, 1913; Clark, 1913–14; Andrus, 1919, and Andrus and Carter, 1922) from carefully prepared stock solutions as shown in table 1, p. 415.

The addition of $MgCl_2$ was compensated by the subtraction of an empirical quantity of NaCl, and similarly any $NaHCO_3$ added in excess of the quantity stated above was compensated by the subtraction of an equimolecular quantity of NaCl.

For calculating the bicarbonate-ion concentration, the ionic activity of solution II was estimated according to Lewis and Randall (1923) and the dissociation of bicarbonate for this ionic activity was taken as 0.73 from the data of Noyes and MacInnes (1920) on KCl.

Since the bicarbonate concentration of the solutions was accurately known, the concentration of carbonic acid in any mixture could be calculated from the hydrogen-ion concentration. The latter was determined with a benzoquinhydrone electrode (Biilmann, 1921) using a clean platinum wire electrode, saturated KCl bridge, saturated KCl-HgCl cell and the usual potentiometric equipment. (For use of quinhydrone elec-

trode see also La Mer and Parsons, 1923; Lewis, 1925; and Biilmann and Kearup, 1924.) Approximately 10 cc. samples were removed from the muscle chamber with a pipette and transferred directly to a Clark electrode vessel containing a small quantity of benzoquinhydrone. The electrode vessel was so filled that when the electrode was in place a small bubble of air, about 0.25 cc., remained. This bubble sufficed to agitate the solution and benzoquinhydrone when the electrode vessel was shaken by the usual mechanical rocker. The platinum wire electrode was cleaned when sluggish by flaming in an alcohol or blue gas flame.

The benzoquinhydrone was prepared by Biilmann's method except that it was further purified by washing with N/10,000 NaOH to remove traces of free acid which seem to be held tenaciously in the sponge-like crystalline precipitate. The washing with NaOH was followed by thorough washing with distilled water. When prepared in this manner the average deviation from the hydrogen electrode around pH 7.0 in

TABLE 1

	I. without $MgCl_2$			II. with $MgCl_2$	
	Stock	Cubic centimeters per liter	Millimols per liter	Cubic centimeters per liter	Millimols per liter
	per cent				
NaCl	20	32.5	111.2	29.5	101.0
KCl	5	6	4.0	6	4.0
$CaCl_2$	5	5	2.2	5	2.2
$NaHCO_3$	ca 5	(6)	(3.5)	(6)	(3.5)
$MgCl_2$	10			10	10.5
Ionic activity					0.1460
γ for uni-univalent salts					0.73

0.0025 molar phosphate and cacodylate solutions did not exceed ± 0.01 pH. With greater buffer content (0.02 molar) no greater deviation from the hydrogen electrode was observed up to pH 7.6. The value 0.4545, obtained by interpolating data given by Biilmann, was used for the benzoquinhydrone electrode when opposed to the saturated KCl-HgCl cell at 22°C.

The denoded atrial strip. All the experiments reported in this paper were performed with the atria of the common fresh water turtle, *Pseudemys elegans.* The heart was excised immediately after pithing the turtle and removing the breast plate. The atrium was cut from the ventricle, as close to the latter as possible, and washed with Ringer's solution at once to prevent coagulation of the blood on the tissue surface. Two or three drops of adrenin hydrochloride solution (1–1000) were added to the Ringer's solution used for washing to prevent the development of "tonus" contractions which will roll the tissue into a knob and make manipulation difficult.

It is well known that the turtle atrium will continue to beat spontaneously and with great regularity for many hours after removal from the body. This rhythmicity has its origin in the sinus tissue adjoining the atrium (Meek and Eyster, 1916; and Schlomovitz and Chase, 1916) and extending into the latter as an expanding fan for a slight distance. For these experiments it was necessary to reduce the tissue to quiescence so that it might be stimulated electrically, or "driven," at any desired frequency. Many substances are known, such as K^+, HCN, etc., which reduce the frequency of contraction, but it was found that all such substances as were tried also decrease the contractility of the muscle and were therefore useless for this purpose. It seems possible that there exists a substance which will arrest the "rhythmic" mechanism without affecting the contractility of the muscle to applied stimuli, but such a substance has not been found.

The desired result was obtained, however, by excising the sinus tissue and its fan-like extension into the atrium, and as a supplementary procedure, adding to the Ringer's solution an appropriate quantity of $MgCl_2$. The excision of the sinus tissue is facilitated by the fact that its junction with the atrial muscle has a characteristic appearance, and is somewhat firmer or tougher than the latter. If the atrium is placed in a small pool of Ringer's solution on a glass plate with the ventricular surface facing the right hand, excision is begun at the atrio-ventricular opening and continued around the base of the sino-atrial junction until all the sinus tissue, including internal connecting bands, is cut free. The excitatory impulse will, as a rule, continue to spread to the atrium until the last connecting bridge between sinus and atrium is cut, after which the sinus will continue to beat while the atrium will remain quiet.

This "denoded" atrium was then opened to the extreme tips by medial incisions along the ventricular surface, and small triangular spreaders of platinum wire were introduced into both ends in such a way as to spread the tissue into a flat, rectangular strip.

In the course of a few minutes such a strip may begin beating spontaneously again, especially if suspended in alkaline Ringer's solution in the presence of adrenin. In such a case inspection will usually disclose a fragment of sinus tissue somewhere near the middle of the strip which can be seen to beat perceptibly before the rest of the muscle. Removal of this sinus fragment will nearly always stop the beating entirely. The "denoded" atrial strip may remain quiescent, despite repeated electrical stimulation and exposure to various chemical conditions, for twenty-four hours or more. In most instances, however, spontaneous contractions appear sooner or later. In view of the well recognized properties of cardiac muscle, it is presumed that the denoded atrial tissue develops, under favorable conditions, a degree of irritability which permits the

formation of ectopic centers of excitation. Spontaneous beating is favored by adrenin, by stretching and by the slight mechanical stimuli afforded by the periodic agitation of the surrounding fluid, while it is abolished by moderate concentrations of CO_2 and by deprivation of adrenin. Neither continuous exposure to CO_2 nor deprivation of adrenin was feasible in these experiments, and it was necessary to find other means of keeping the tissue quiet. Magnesium salts are known to have an anesthetic effect in general (for the action of strong $MgCl_2$ solution on the frog heart, see Burridge, 1918–20b), and it was found that the addition of $MgCl_2$ to the Ringer's solution completely repressed all tendency towards spontaneous beating (except in very rare instances) without affecting to any degree the contractility of the muscle under artificial stimulation. Since the addition of $MgCl_2$ in the same concentrations has no effect on the rhythmicity of the atrium with sinus tissue intact, it is inferred that its action on the denoded atrium results from a lowering of the general irritability of the tissue rather than from any direct influence on the mechanism underlying the phenomenon of rhythmic discharge.

The so-called "tonus" contractions of cardiac tissue. The slow, recurring "tonic" contractions which were first described by Fano (1887) and which are shown by most excised turtle atria, are familiar to every student of physiology. Botazzi (1897) ascribed these waves to tonic changes in the sarcoplasm (see also Porter, 1905, and Gesell, 1916), while Rosenzweig 1903) attributed them to contractions of smooth muscle fibers directly beneath the atrial endothelium which he first described and which he regarded as a continuation of the tunica media of the great veins. Rosenzweig's explanation of these "tonus waves" has met with no adequate refutation (see Andrus, 1919, and Snyder and Andrus, 1919) and his explanation is accepted here. It is an interesting fact that these waves persist in their usual form in the quiescent, denoded atrial strip. In $MgCl_2$-$NaHCO_3$-Ringer's solution at pH 8.0 to 8.5, in the absence of adrenin, they appear recurrently at irregular intervals, whether the atrial strip is being made to contract in consequence of electrical stimulation or not. Neither starting nor stopping the electrical stimulation seems to have any effect on the duration or course of the tonus contractions, a fact in keeping with Rosenzweig's explanation.

It is well known that adrenin in relatively large doses abolishes these tonus contractions, and since they would completely defeat the use of atrial strips for quantitative observations, it is necessary to keep the preparation well adreninized at all times. To this end 4 to 8 drops of 1–1000 adrenin hydrochloride solution were added to the heart chamber (370 cc.) each time the latter was filled with a fresh solution.

Gaskell (1880–82) found that alkali increased the tonus waves in frog hearts, and Burridge (1913, 1921), Dale and Thacker (1913–14), and Andrus (1919) have observed

similar effects. It has been noted in this series of experiments that alkali (pH 9.0-11.0) has this effect even in the presence of excessive quantities of adrenin. Both the rapidity of onset and the amplitude of the waves are increased by increasing alkalinity. The effect is the same whether $NaHCO_3$ is present or not, and it is perfectly reversible when the tissue is returned to a less alkaline (pH 8.0) solution.

In the absence of adrenin, the waves make their appearance very rapidly in Ringer's solution as it is usually prepared with $NaHCO_3$ (pH 8.0 to 9.0), but they are rapidly abolished by increasing the concentration of CO_2. Gaskell (1880-82) observed that lactic acid-salt solutions promoted increased relaxation of frog hearts; similarly "increased relaxation" of cardiac muscle, without particular reference to recurrent "tonus waves," has been observed after increasing the acidity of $NaHCO_3$-Ringer's solution by Mines (1913c), Burridge (1913), Andrus (1919) and others. To what extent this "increased relaxation" is dependent on the smooth muscle fibers is an important, but open, question because of its bearing on the possible existence of a state of true tone in isolated cardiac muscle. With the atrial preparation described above, whether denoded or not, no definite evidence of such tone has been obtained. The adrenalinized atrial strip shows no significant spontaneous changes in diastolic level, except under conditions which may reduce the rate of relaxation (such as high concentrations of CO_2). After several hours of varied chemical manipulation, the diastolic level will gradually rise, but this rise appears to be irreversible and suggests the progressive development of permanent changes in the muscle which prevent complete relaxation, or the development of a death rigor.

Influence of rate on contractility. It was discovered by Bowditch (1871) that an effective electrical stimulus, regardless of its strength, produces in cardiac muscle the greatest contraction that can be produced by any strength of stimulus in the condition of the muscle at the time (all-or-none law). Mines (1913a) subsequently showed that in the ventricle of the Selachian fish, *Torpedo*, an artificial stimulus, at a short interval after the normal beat, produces a greater response than the normal one (see also Adrian, 1920, and Wastl, 1922).

That there exists a very important relation between the contractility and the frequency at which the muscle contracts may be demonstrated on the denoded atrial strip. There is an optimal frequency, roughly between 3 and 10 seconds, at which the contractility is maximal; as the frequency is either increased or decreased from this optimum, the contractility falls off, more rapidly with increased than with decreased frequency. As an illustration, the following figures were obtained by driving an atrium at various intervals: 1, 2, 3, 5, 6, 10, 15, 30, 60 seconds and 5 minutes, amplitude of isotonic myogram: 5.5, 5.8, 6.6, 6.8, 6.7, 6.4, 5.8, 5.6, 5.2 centimeters, respectively. • After a change from one frequency to another the contractility gradually changes from the old equilibrium level to the new; this transition appears to be effected not in relation to time, but in relation to the number of contractions, 60 to 100 contractions being required to establish a new level either higher or lower than the preceding one. Thus if the interval is decreased from 60 seconds to 2 seconds, equilibrium may be reached in 2 to 3 minutes, but if the change is from 5 minutes to 60 seconds, an hour or more may be required.

This important relation between frequency and contractility may take various forms according to the circumstances. Thus, if an atrium which has been beating at a rate within the optimum frequency, and therefore with maximal amplitude, is allowed to rest, the initial contractions after the rest period will be reduced, and the subsequent contractions will progressively increase until the equilibrium level is again reached. This is the familiar phenomenon of "treppe." If, on the other hand, the atrium is allowed to rest after beating at frequencies greater than the optimum, the rest period is followed by contractions which are abnormally great and which are progressively reduced to the equilibrium level, thus giving what may be called an "inverted treppe." Mines interpreted the phenomena of treppe in terms of lactic acid production and an optimal hydrogen-ion concentration in certain regions of the muscle cell. The phenomena do suggest that there is some reactant in the muscle cell on the concentration of which, perhaps in some localized region, the development of tension is intimately dependent; and that the concentration of this reactant is affected by the act of contraction itself. But that lactic acid and the hydrogen-ion are not the determining factors is shown by the facts that these phenomena are not essentially modified in the state of reduced contractility which follows oxygen lack (when it is known that there is an accumulation of lactic acid in the muscle), in solutions saturated with oxygen, or under high or low concentrations of CO_2.

Effect of CO_2 on rhythmicity. Though nearly all the experiments reported here were performed on denoded atria which were driven at desired rates by electrical stimulation, this opportunity will be taken to comment briefly on the effects of CO_2 and HCl on the rate of contraction of excised but naturally beating atria. Many experiments of this kind were performed in the preliminary survey of this problem which will not be described here. In these experiments the Ringer's solution described as no. 1 was used, with sufficient adrenin to prevent smooth muscle contractions. It was observed that the rhythmicity is maintained with remarkable constancy under the most severe chemical conditions. The concentration of CO_2 can be raised to a degree which completely abolishes the contractility; yet the last perceptible twitches as the muscle settles into apparent quiescence, and the first perceptible twitches as it recovers after reduction of the CO_2 concentration, occur at a frequency which is practically the same as that in alkaline Ringer's solution. Similarly, the muscle may be placed in bicarbonate-free Ringer's solution to which sufficient HCl has been added to produce an acidity of pH 2.8 to 3.0; under these conditions the contractility is rapidly diminished to zero, yet no large change in frequency occurs. In some instances, particularly in preparations which have been in use for several hours, a change of frequency from 35 to 25 per minute, or a change of that order may be observed under one condition or another, but such slight and inconstant changes can hardly be considered of prime significance. Several observers have commented on changes in rhythm in perfused or isolated hearts associated with changes in hydrogen-ion concentration (see Herlitzka, 1912; Clark, 1913-14; Mines, 1913c; Andrus, 1919 and Burridge, 1920). Mansfeld and Szent-Györgyi (1920) believe that CO_2 is a natural stimulus provoking rhythmic automaticity in the heart. Andrus

(1924) has emphasized differences in the hydrogen-ion concentration of the cell and the surrounding fluid as determining factors in this rhythmic activity. Bainbridge (1923), in refutation of Mansfeld's and Szent-Györgyi's conclusions, showed that the frog's heart maintains its activity and rhythm in solutions at pH 7.7 which are devoid of CO_2 or bicarbonates. Mines (1913b) showed that mechanical movement of the heart may be completely abolished by alteration of hydrogen-ion concentration while regular spontaneous electrical variations of normal form and extent continue, and similar observations were made by Dale and Thacker (1913–14). In view of this evidence, it seems improbable that the hydrogen ion or any other component of the acid-base system plays a direct or important part in originating or maintaining this rhythmic activity. Such minor changes in rhythm as can be produced by alteration of the acid-base components must be attributed to secondary influences on the rhythmic mechanism, effected indirectly through changes in conductivity, irritability, or other associated physiological properties of the tissue.

Oxygen supply. The need for oxygen in the recovery process in turtle cardiac muscle is shown by Snyder (1922), and by observations made by Doctor Redfield (personal communication) in this laboratory, who has found that the absence of oxygen leads to a loss of contractility and the accumulation of lactic acid in the muscle.

The use of CO_2-air mixtures bubbling through the solution bathing the muscle is not feasible in experiments where a variety of changes in CO_2 concentration must be quickly effected. In the light of many experiments it was concluded that sufficient oxygen is supplied if the muscle is placed in a relatively large volume of solution (300 to 400 cc.) previously saturated with air. Under these conditions, if the solution is adequately circulated in the muscle chamber, no evidence of oxygen deficiency appears in 3 to 4 hours. Since the majority of experiments require only 30 minutes' to one hour's confinement in a particular solution, it is believed that the oxygen supply is entirely adequate and without effect upon the experimental results.

The action of CO_2 on the contractility of atrial muscle. When a denoded atrial strip, immersed in $NaHCO_3$-Ringer's solution at pH 8.0 to 9.0, is stimulated at 5-second intervals it gives a series of isotonic contractions of uniform and very constant amplitude. If a quantity of this same $NaHCO_3$-Ringer's solution, saturated with CO_2, is mixed with the solution surrounding the tissue, the contractility of the muscle is rapidly diminished, falling in 4 to 5 minutes to a new level. This depressing action of CO_2 and the general magnitude of the effect on the isotonic myogram are illustrated in figure 1, where the amplitude of the isotonic myogram in centimeters, H, is plotted against time in minutes after the addition of CO_2. (The experiments illustrated in this figure will be described later.) The greater the quantity of CO_2 added, the greater is the loss, or decrement, in contractility.

This effect of CO_2 in decreasing the contractility might be due to one

or several of the following factors acting directly on the contractile mechanism:

A. The increased *hydrogen-ion* concentration of the fluid.

B. The *physical* properties of molecules of CO_2 or molecules of H_2CO_3.

C. The increased *intracellular* acidity (occasioned by the penetration of CO_2 and the subsequent intracellular liberation of hydrogen ions).

D. The accumulation of *lactic acid* (independent of its acid properties), in consequence of its production during the contractile act and a reduced rate of removal occasioned by increased intracellular acidity.

The rôle of the hydrogen-ion concentration of the fluid (A) in this reduction of contractility is open to direct examination. The atrial strip will contract (either spontaneously or after stimulation) in Ringer's solution containing no $NaHCO_3$, only traces of CO_2, and no other weak acids. If such a solution is brought by the addition of HCl from pH 8.0 to pH 6.0 (the greatest acidity involved in the CO_2 experiments) the contractility is not affected to any marked degree. There is a gradual and progressive loss in contractility, amounting to about 0.5 cm. in the isotonic myogram in one hour. (Detailed experiments dealing with the effects of HCl will be described later.) Since a *rapid and large decrement* results from an increase in concentration of CO_2 in the fluid and since *no such decrement* results from the concomitant increase in concentration of hydrogen ions alone, it is concluded that the change in hydrogen-ion concentration in the fluid is not the cause of the decrement observed with CO_2.

The action of CO_2 might be a physical one peculiar to molecules of either CO_2 or H_2CO_3, analogous to the action of alcohol or ether (B); or this action might be a chemical one, attributable to the capacity of CO_2 to penetrate the tissue and participate in the intracellular acid-base equilibria (C and D). If the first circumstance obtained, the same effect should be produced by the same concentration of CO_2 or H_2CO_3, or by the same change in concentration, regardless of the concentration of bicarbonate ions. (This is true of the action of benzoic and salicylic acids in repressing cell division in marine eggs, Smith, 1925.) If the second circumstance obtained, then the same effect should be produced by the same intracellular hydrogen-ion concentration, which in the presence of different concentrations of bicarbonate ions would not be dependent solely on the concentration of CO_2. It is possible, of course, that both these circumstances might be operative at the same time. It may be said that the effects of CO_2 are not solely determined by the concentration of CO_2, but are influenced by the concentration of bicarbonate ions in the direction and to the degree to be expected if these effects are due to its acid properties. It is inferred from this evidence, therefore, that the effects of CO_2 in this instance may be analyzed on the premise that its action is due solely to

its acid properties, and since no independent evidence is available, the correctness of this premise can only be tested by the success of this analysis.

Hartree and Hill (1923–24) have shown that an increase in CO_2 tension reduces the rate of recovery in a fatigued skeletal muscle, the recovery process involving principally the oxidation of part of the lactic acid formed during contraction and the conversion of the rest into glycogen (Meyerhof, 1924). It is to be expected that the cycle of chemical events which accompanies the act of contraction in skeletal muscle would be qualitatively duplicated in cardiac muscle, but that in the latter the time relations would be altered in ways favoring complete recovery despite the frequent repetition of the contractile act (cf. Snyder, 1922). It is conceivable, therefore, in view of Hartree and Hill's observations, that the increased acidity in the muscle might lead to the accumulation of lactic acid which in time might reduce the contractility by virtue of reversible participation in the contractile process (D). On the other hand if lactic acid had no such specific effect, or did not accumulate to an appreciable degree, then the effects of CO_2 must be considered an immediate consequence of increased intracellular acidity (C). Since lactic acid is formed during or in consequence of the contractile act, it is possible by comparing the effects of CO_2 on atria which are essentially at rest and contracting at different rates, to determine to what degree these effects are due to the accumulation of lactic acid in the muscle.

The conclusion drawn from such experiments is that so long as the muscle is contracting at frequencies less than a critical value, which is about once every 5 seconds, the effects of CO_2 are principally, if not entirely, due to the increased intracellular acidity resulting from increased intracellular carbonic acid; because,

1. Loss of contractility occurs when the concentration of CO_2 is increased, whether the muscle is contracting frequently or at widely separated intervals.

2. The decrement in contractility produced by an increment in CO_2 concentration between particular limits is independent of the frequency of contraction.

3. When the CO_2 concentration is either increased or decreased, the time required to reach a new level of contractility is essentially independent of the immediate frequency of contraction. (The time required to reach this new level is obviously the time required for CO_2 to enter or leave the muscle; it is, as would be expected, not only independent of the frequency of contraction but essentially independent of the change in concentration of CO_2.)

Experiments demonstrating these points are illustrated in figure 1. In these experiments $[H_2CO_3]$ was increased from 1.2 to 3.8–4.0 millimols per liter in successive experiments with one atrium stimulated at 5-, 30-

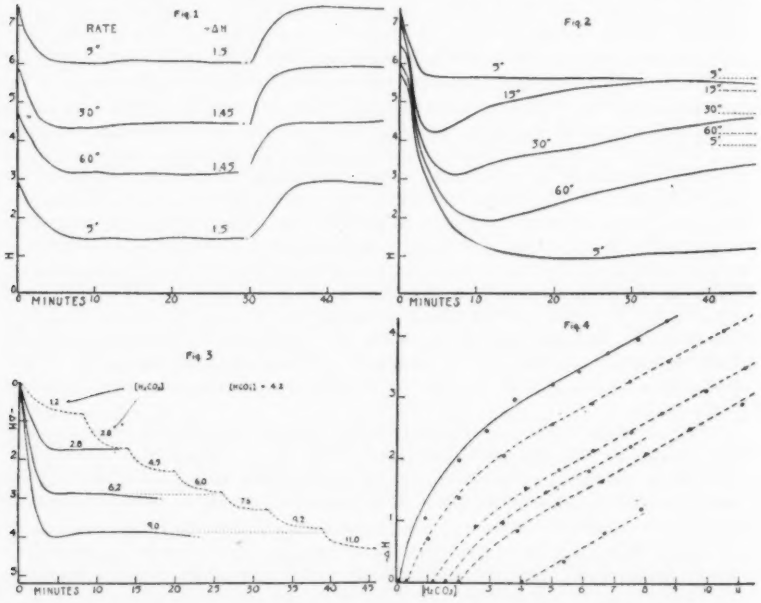
and 60-second and 5-minute intervals. (In this and all other figures the height of the isotonic myogram, H, is expressed in centimeters and the loss in contractility, or decrement, is designated by $-\Delta H$. The graphs are reconstructed from careful measurements of myograms.) The fact that the contractility varies considerably at different frequencies accounts for the differences in the initial contractility in these experiments.

It should be noted, however, that under certain conditions lactic acid may accumulate, and by virtue of its acid properties, contribute to, or otherwise complicate the action of CO_2. Although there is a tendency for the establishment of an equilibrium level after an increase in CO_2 concentration, this level cannot be maintained indefinitely, particularly at high concentrations of CO_2. Beginning 20 to 40 minutes after the initial fall, there is a slow and progressive loss in contractility which continues indefinitely and which, it seems probable, is due to lactic acid accumulation. The more rapidly the muscle is beating, the sooner this secondary, progressive loss in contractility makes its appearance. In fact, if the muscle is contracting once every $1\frac{1}{2}$ to 3 seconds, a new level is never reached under moderate or high CO_2 concentrations, but the contractility continues to decrease at a diminishing rate for several hours. In this case the process is closely described, after the first two or three minutes (i.e., after equilibrium has been reached with respect to CO_2), by the equation $dH/\log C = \text{constant}$, where H = height of isotonic myogram and C = number of contractions. It seems possible that this logarithmic relation can be explained by the assumption that the quantity of lactic acid furnished by each contraction is determined by the amplitude of that contraction, which is in turn determined by the total lactic acid (i.e., more directly by the hydrogen-ion concentration) present in the muscle.

Further evidence in this direction is afforded by some unpublished experiments of Doctor Redfield's which he kindly permits me to mention. In the absence of oxygen, atrial tissue undergoes progressive loss of contractility which parallels, and apparently results from the accumulation of lactic acid; the decrement is logarithmically related to the number of contractions, the isometric contractility diminishing by about $\frac{1}{300}$ of its value with each successive contraction. Under anaerobic conditions it would be expected that practically all the lactic acid produced by contraction would accumulate in the muscle; yet under these conditions the decrement is only a small fraction of the total contractility ($\frac{1}{300}$), whereas the contractility may be nearly abolished by CO_2 in 5 to 10 minutes, whatever the rate of contraction. This fact bears out the conclusion reached above that the decrement observed under CO_2 is not a consequence of lactic acid accumulation but due immediately to increased intracellular acidity.

Anomalous phenomena. It has been noted that when certain atria are subjected to an abrupt increase in CO_2 concentration, instead of showing a reduction in contractility to a new and constant level, they show an excessive depression (under-shooting) which is followed by a gradual return of contractility to approximately the expected level. An extreme instance of this kind is illustrated in figure 2. This phenomenon of under-shooting is slight or entirely absent when contraction occurs every five seconds, but becomes progressively greater as the frequency of contraction diminishes. Moreover, the slower the frequency of contraction, the slower is the rate of recovery to the final equilibrium level; it appears that the recovery from the under-shooting is a function of the number of contractions, rather than a function of time (cf. readjustment of contractility after change from one frequency to another, described above p. 419).

The facts that the under-shooting is absent if the frequency falls within the range of maximum contractility, i.e., 3 to 10 second intervals, and that it becomes increasingly augmented as the frequency is further decreased from the optimum, suggest that this phenomenon is intimately related to the factor or factors that underlie the previously described changes in contractility with changes in frequency. The general picture suggests that there is some factor or process which *favors* contractility, the effective presence of which is determined by the *frequency* of contraction; and that in certain muscles, or perhaps in all muscles under the appro-



The contractility of atrial tissue is expressed in all figures in centimeters height of the isotonic myogram. Since the decrement in contractility produced by a definite change in concentration of CO_2 is essentially independent of the maximal contractility, and since the latter may vary considerably in successive experiments with one atrium, the experimental results are for the most part expressed in terms of decrement, $-\Delta H$. All concentrations are expressed in millimols per liter. Figure 1 is described in the text on pages 420 and 425; figure 2, p. 423; figure 3 and figure 4, p. 426.

priate conditions, this factor can be momentarily displaced by abrupt changes in CO_2 concentration. There is a suggestion that this hypothetical factor involves calcium, for under-shooting is sometimes entirely abolished, and always greatly reduced by raising the calcium content of the solution from 2.2 to 6.6 millimols per liter. With increased calcium, the contractility is usually considerably increased, but the variation of amplitude with frequency and the effects of rest periods (treppe) are not significantly modified (cf. Burridge, 1918-20a and b). The increased contractility which results from an increase in calcium suggests that the Ringer's

solution described may be deficient in this salt for turtle heart muscle. On the other hand increasing the $CaCl_2$ in the solution, with or without an increase in KCl, is not altogether beneficial for it results frequently in loss of irritability and loss of resistance to CO_2, particularly at high concentrations.

So far as the bearing of under-shooting on the present problem is concerned, it may be stated that even those atria which show under-shooting at low frequencies (once a minute) do not as a rule show it at frequencies within the optimum range (3 to 10 seconds) and experiments made within this range do not appear to be complicated by it. When under-shooting does appear at 5-second intervals, it is likely to become worse after repeated exposures to CO_2 and the muscle has to be discarded. Frequently the use of a particular tissue is terminated after a short time by this complication.

Recovery from exposure to CO_2. The process of recovery when the atrial strip is transferred from a high to a low CO_2 concentration is divisible into two distinct phases: a rapid recovery phase, requiring 4 to 5 minutes, in which the contractility returns nearly to normal (see fig. 1); and a subsequent phase, requiring 20 to 60 minutes, in which the contractility may gradually increase to a maximum and subsequently fall to a slightly lower and constant level. The first phase clearly represents a period during which CO_2 is escaping from the tissue, since it has the same time relations as the reverse process of penetration. The second phase, which is variable in a single tissue, suggests a readjustment of other factors in the muscle which have been displaced by the exposure to CO_2.

Exposure to CO_2, with the resulting reduction in contractility, appears to effect no permanent injury to the muscle. One atrium can be subjected to many separate treatments with CO_2 without serious impairment. There is, of course, a gradual and progressive loss in contractility, the extent of which varies with different atria, and with the severity of the chemical manipulation, but the fact that some atria will survive repeated use for 24 hours with an insignificant loss in contractility shows the essential reversibility of the action of CO_2. The use of a particular atrium is more frequently terminated by the appearance of under-shooting or other anomalous phenomena than by the outright exhaustion of the tissue.

Attempts to keep atria in good condition over night have not been particularly successful. It has been found to be expedient, if constancy in response is desired, to work continuously with a muscle for as long a period as possible. In brief, it may be said that the exhaustion of contractility occurring in the course of a 12 or 15 hour rest period is just about as great as the exhaustion occurring during that period under continuous experimental manipulation, and from all considerations it is best to get as many quantitative results as possible while the tissue is fresh.

The constancy of the action of CO_2 on atrial tissue. Quantitative observations on atrial tissue are simplified by the fact that the decrement in contractility produced by a definite change in concentration of CO_2 is prac-

tically independent of the maximal contractility. This fact is brought out by the experiments shown in figure 1 and in several subsequent experiments. The maximal contractility of any one tissue will vary in the course of time, since there is a gradual loss of contractility *in vitro* which is more or less accelerated by repeated chemical manipulation; yet constancy of decrement is maintained for 24 hours or more. The height of the isotonic myogram can be varied by varying the weight on the muscle lever through wide limits without disturbing this constancy of decrement; one of several experiments in which this factor was examined gave the following results for an increase in $[H_2CO_3]$ from 0.6 to 2.0 millimols per liter ($[HCO_3^-]$ = 2.5 millimols per liter): H = 8.0, 7.2, 6.7, 6.0, 5.6, 5.0, 4.8; $-\Delta H$ = 1.25, 1.20, 1.26, 1.30, 1.18, 1.22, 1.18 respectively. In view of the variation of maximal contractility which occurs in successive experiments, and of the fact that the decrement is the constant feature of the action of CO_2, the experimental results are for the most part given in terms of decrement ($-\Delta H$).

When the contractility is reduced below 2.0 or 1.5 cm. by large concentrations of CO_2, the mechanical limitations of the recording apparatus will obviously lead to aberrant results; an equilibrium level is not well maintained under these conditions, and the constancy of decrement described above no longer holds true.

The process of recovery to constant contractility after exposure to CO_2 consumes more time than any other procedure. It is expedient, therefore, to make a series of observations at progressively increasing CO_2 concentrations in one experiment, allowing sufficient time after each increase for the contractility to reach a constant value. This seriatim method not only saves time and makes possible a number of experiments with one tissue, but it reduces to a minimum the possible sources of error in each series of observations. An experiment of this kind is shown in figure 3. In this instance, after the initial seriatim experiment, 3 single determinations were made using different quantities of CO_2. The results show that the decrement corresponding to a certain increment in CO_2 concentration is the same whether this increment is added all at once or in several successive portions.

$-\Delta H$ as a function of $[H_2CO_3]$. If the total decrement, $-\Delta H$, is plotted against $[H_2CO_3]$, the resulting curve is distinctly rectilinear except near the origin where a very rapid decrement may result from slight increases in $[H_2CO_3]$. The extent of this "curvilinear" portion of the curve varies considerably in different atria; it may be wholly absent in some atria, and when present, it may become more marked in solutions of high bicarbonate-ion concentration. An extreme example of the curvilinear type is illustrated in figure 4. In these experiments a number of seriatim determinations were made on one atrium at the same bicarbonate-ion concen-

tration, but differing in respect to the initial concentration of CO_2. There are two significant features shown by these experiments; first, each curve after an abrupt rise levels out and becomes a straight line at higher CO_2 concentrations; and, second, the dotted curves are all sections of the solid curve intercepted at progressively higher CO_2 concentrations. The latter fact is simply a consequence of the circumstances that the muscle behaves in exactly the same manner (i.e., follows a fundamental curve) in successive experiments, and that the successive experiments begin at progressively higher points on this fundamental curve.

In the following analysis of the action of CO_2 it will be necessary to determine with the greatest possible exactness the relative "physiological effectiveness" of carbonic acid in various bicarbonate solutions. It is apparent that differences in the early portions of curves relating $-\Delta H$ to $[H_2CO_3]$ are not significant if we consider as essential only the rectilinear relation, which obtains for all atria and in all solutions at relatively high CO_2 concentrations. The significant feature of the rectilinear relation is, of course, the slope. This slope can be determined directly from the experimental data by averaging the ratio $d[H_2CO_3]/d(-\Delta H)$; and from this slope the concentration of carbonic acid required to produce a given decrement can be calculated for a family of hypothetical, rectilinear curves having a common origin at zero values of $[H_2CO_3]$ and $-\Delta H$. (The same result could be obtained experimentally by beginning all experiments at relatively high CO_2 concentration, but such restriction of experiment and observation is patently less desirable than the simple deletion of data at low CO_2 concentrations.)

The action of CO_2 in terms of intracellular acidity. The rôle of the bicarbonate ion in the action of CO_2 can be determined by making seriatim determinations as described above in solutions having different bicarbonate concentrations. A series of such experiments on one atrium is summarised in figures 5 and 6 and table 2. In these experiments the atrium was left for one hour in each new bicarbonate solution to insure equilibrium with the latter, and a preliminary dose of CO_2 was given in each case to facilitate equilibrium.

It will be observed from figure 5 that as the concentration of bicarbonate ion in the fluid increases, CO_2 becomes progressively less effective in reducing the contractility. As an index of the physiological effectiveness of carbonic acid in the various solutions we may take the concentration required to produce a given decrement, calculated in the manner described in the preceding section from the slopes of the curves. These slopes are given in table 2 under the heading $d[H_2CO_3]/d(-\Delta H)$ and plotted in figure 6 against the concentration of bicarbonate ions in the fluid. The interpretation of these results relative to changes in the con-

centration of bicarbonate ions in the cell is based on the following propositions:

a. The action of CO_2 is attributable solely to its acid properties (proposition C, pages 421 and 422).

TABLE 2

$[HCO^-_3]$	p[H]	$[H_2CO_3]$	H	$-\Delta H$	$\frac{d[H_2CO_3]}{d[-\Delta H]}$	$(H^+) \times 10^7$	$\frac{d(H^+) \times 10^7}{d(-\Delta H)}$
0.635	7.27	0.11	6.00			0.1	
	6.67	0.41	5.62	0.38	(0.805)	0.39	(0.763)
	6.16	1.35	4.72	1.28	1.04	1.28	1.0
	5.9	2.42	3.70	2.30	1.04	2.30	1.0
	5.79	3.17	2.96	3.02	1.04	3.0	1.0
	5.70	3.92	2.10	3.90	0.81	3.7	0.76
				mean	0.98		
				check	1.26		
1.27	7.96	0.04	8.35			0.03	
	6.83	0.58	7.9	0.45	(1.20)	0.46	(0.95)
	6.45	1.38	7.3	1.05	1.33	1.1	1.07
	6.24	2.25	6.76	1.59	1.61	1.8	1.30
	6.12	2.97	6.28	2.07	1.50	2.38	1.21
	6.05	3.50	5.78	2.57	1.06	2.79	0.82
	5.97	4.18	5.26	3.09	1.31	3.35	1.08
	5.92	4.70	4.88	3.47	1.37	3.74	1.02
	5.86	5.20	4.48	3.87	1.25	4.16	1.05
	5.83	5.80	4.00	4.35	1.25	4.61	0.94
				mean	1.33		
				check	1.33		
2.54	7.24	0.45	6.00			0.27	
	6.55	2.20	4.90	1.10	(1.59)	1.34	(1.26)
	6.39	3.18	4.25	1.75	1.51	1.94	0.92
	6.27	4.22	3.75	2.25	2.08	2.57	1.26
	6.21	4.82	3.38	2.62	1.62	2.94	1.00
	6.15	5.54	2.91	3.09	1.53	3.38	1.19
	6.09	6.37	2.50	3.50	2.02	3.86	0.94
	6.03	7.32	1.95	4.05	1.72	4.45	1.07
	6.00	7.88	1.65	4.35	1.66	4.76	1.03
				mean	1.73		
				check	1.83		
4.24	7.66	0.29	6.68			0.28	
	6.84	1.88	5.80	0.88	(1.81)	0.87	(0.66)
	6.55	3.68	5.05	1.63	2.40	1.7	1.12
	6.43	4.82	4.55	2.13	2.28	2.23	1.06
	6.31	6.38	3.85	2.83	2.22	2.94	1.01
	6.23	7.65	3.30	3.33	2.54	3.54	1.20
	6.18	8.60	2.90	3.73	2.38	3.96	1.05
				mean	2.36		
				check	2.28		

TABLE 2—*Concluded*

$[HCO^-_3]$	p[H]	$[H_2CO_3]$	H	$-\Delta H$	$\frac{d[H_2CO_3]}{d[-\Delta H]}$	$(H^+) \times 10^7$	$\frac{d(H^+) \times 10^7}{d(-\Delta H)}$
5.9	7.4	0.725	5.25			0.27	
	6.83	2.73	4.30	0.95	(2.12)	1.02	(0.75)
	6.57	4.98	3.59	1.66	3.17	1.86	1.18
	6.45	6.55	3.07	2.18	3.02	2.44	1.11
	6.36	7.98	2.45	2.80	2.75	2.98	1.04
	6.28	9.65	2.00	3.25	3.71	3.60	1.38
				mean	2.96		
				check	2.88		
7.65	7.4	0.95	6.1			0.29	
	6.96	2.59	5.38	0.72	(2.30)	0.80	(0.72)
	6.73	4.39	4.8	1.30	3.10	1.36	0.97
	6.57	6.37	4.3	1.80	3.96	1.98	1.24
	6.47	7.90	3.86	2.24	3.41	2.46	1.09
	6.42	8.89	3.50	2.60	2.75	2.79	0.91
	6.36	10.2	3.26	2.84	5.45	3.18	1.62
	6.31	11.5	2.86	3.24	3.25	3.58	1.00
	6.25	13.5	2.30	3.80	3.57	4.16	1.03
				mean	3.65		
				check	3.43		

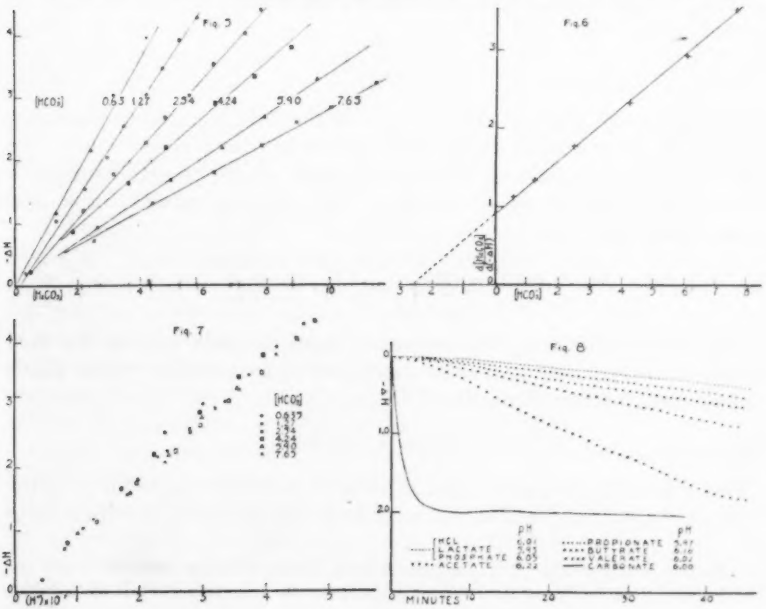


Figure 5 is described in text on p. 427; figure 6, p. 427; figure 7, p. 432; figure 8, p. 433.

b. The site of action of CO_2 is here defined as "intracellular," in view of the fact that the effects characteristic of CO_2 and attributed to its acid properties, are not produced by a concomitant increase in the hydrogen-ion concentration of the fluid.

Since for a particular bicarbonate solution, the decrement in contractility, $-\Delta H$ (figured from the maximal contractility when [HA] = O) is *de facto* constant for a particular increment in [HA], it follows from (a) and (b) that particular values of $-\Delta H$ correspond to particular values of (H^+). Therefore we must examine the relations obtaining between the carbonic acid and bicarbonate ion concentrations required to produce particular decrements in contractility.

c. The concentration of carbonic acid is taken equal in fluid and cell. The theoretical basis for this proposition has been discussed in a previous paper (Smith, 1925) and will not be reviewed here. The dissociation constant of carbonic acid in the cell is likewise assumed to be the same as it is in the fluid. Using *round brackets* to designate intracellular concentrations and *square brackets* to designate concentrations in the fluid,

$$(HA) = [HA] \tag{1}$$

It follows from equation 1 and from the mass law,

$$H^+ = \frac{HA}{A^-} k \tag{2}$$

that the concentration of carbonic acid in the fluid required to produce a particular decrement in contractility varies in direct proportion to the concentration of bicarbonate ions in the cell. Using numeral suffixes to denote particular values of variables, the condition obtains for a particular decrement that

$$(H^+)_1 = (H^+)_2 = \frac{[HA]_1{}^k}{(A^-)_1} = \frac{[HA]_2{}^k}{(A^-)_2} \tag{3}$$

The equation relating the concentration of carbonic acid in the fluid required to produce a particular decrement in contractility to the bicarbonate-ion concentration in the fluid (fig. 6) is

$$[HA] = x([A^-] + a) \tag{4}$$

where x is a proportionality factor having a particular value for a particular decrement,[1] and a is an intercept or a "fixed" constant with a value

[1] It happens, because of the approximately linear relation between $-\Delta H$ and [HA] that x has the same value for all decrements, but this fortuitous relation has no significance for the present argument.

of 0.0028 in terms of $[A^-]$, expressed in mols per liter. Substituting for [HA] in (3) by (4),

$$\frac{(A^-)_1}{(A^-)_2} = \frac{[A^-]_1 + a}{[A^-]_2 + a} \tag{5}$$

That is, the rate of increase of (A^-) is related by simple porportion to the rate of increase of $[A^-]$; we may therefore write the rectilinear equation:

$$(A^-) = r[A^-] \pm y \tag{6}$$

(The terms r and y in this equation should not be confused with x and a in (4); (4) is an experimental statement while (6) is an inductive relation synthesized from (5).)

Substituting for (A^-) in (3) by (6) and inserting observed values for [HA] and $[A^-]$ in mols per liter, it follows that

$$y = + r0.0028 \tag{7}$$

Substituting for y in (6) and writing α for the numerical term 0.0028,

$$(A^-) = r([A^-] + \alpha) \tag{8}$$

Equation 8 has been derived for a particular intracellular hydrogen-ion concentration (particular decrement in contractility). By performing similar operations for any other intracellular hydrogen-ion concentration (any other decrement), equations similar to (5), (6), (7) and (8) will be obtained, containing new constants which can be designated as prime;

$$(A^-) = r'[A^-] \pm y' \tag{6a}$$

$$y' = + r'0.0028 \tag{7a}$$

$$(A^-) = r'([A^-] + \alpha) \tag{8a}$$

The term α is commonly designated in the mathematical sense as a "fixed" or "absolute" constant; this term expresses an apparent tendency on the part of the cell to pile up a quantity of anion in excess of that in the fluid, in terms of the proportionality factor, r. It should be noted, however, that if r were very small and $[A^-]$ less than α, (A^-) would actually be less than $[A^-]$, when the term "excess" would be misleading. It seems preferable, therefore, to speak of α as designating the "fixed" anion in the cell, in contradistinction to the variable term $r[A^-]$.

The values of the proportionality factors r and r' cannot be determined from the experimental data, and they may or may not have the same value. All that these data show is that for a particular intracellular hydrogen-ion concentration, (A^-) increases in simple porportion to $[A^-] + \alpha$, but the value of the proportionality factor must be determined by independent means. It appears from theoretical considerations that a condition of unequal distribution of anions between cell and fluid may arise in con-

sequence of the presence in the cell of non-diffusible ions (Donnan effect). It seems that this principle of unequal distribution, by reason of its independent origin, would be operative regardless of the manner in which a penetrating anion gains access to the cell. Though the Donnan law cannot apply in its usual form, $(A^-)/[A^-] = R$, (which is incompatible with equation 8) it remains possible that it may apply within the special terms of this equation. If so, it would be expected that the condition of unequal distribution would operate on all increments of the "penetrating" anion and since α has the same denomination as $[A^-]$, it must also be multiplied by R to give it the denomination of (A^-); thus

$$(A^-) = R([A^-] + \alpha) \tag{9}$$

The term r in equation 8 has been previously referred to as a "Donnan ratio" (Smith, 1925). The conflict between equation 8 and the usual form of the Donnan law makes this terminology inadvisable; it is preferable to say that, while r is a mathematical consequence of the experimental data, it may simultaneously express the operation of the Donnan principle, if, as we suppose, this principle is operative under these conditions.

By way of testing the general coherence of the experimental data in respect to propositions a, b and c and equation (8), we may summarize the data given in figure 6 and table 2 in terms of intracellular hydrogen-ion concentration, calculated by combining (1) and (8):

$$(H^+) = \frac{[HA]k}{r([A^-] + \alpha)} \tag{10}$$

(In view of the fact that lactic acid is intermittently being formed during contraction and removed during the subsequent recovery period, and that other factors may be operating to cause momentary deviations from a constant condition, it is preferable to construe the above equation as indicating a virtual, rather than a real concentration of hydrogen ions in the cell.)

The value of r in this equation is unknown; but if we think of r as expressing a possible Donnan effect it would, from theoretical considerations, be expected to remain constant for a particular intracellular hydrogen-ion concentration (particular decrement); and we may for the purposes of this calculation assign it a value of unity. Should its value be as great as 1.3 or as low as 0.7 (the probable upper and lower limits) p(H) would only be altered by ± 0.15. The values for the virtual intracellular hydrogen-ion concentrations which are given in table 2 were calculated on the basis that $r = 1.0$ and $\alpha = 2.8$ millimols per liter. The values are shown graphically, plotted against $-\Delta H$, in figure 7. The only significant feature in this curve is the approximate agreement of the separate bicarbonate experiments at particular values of (H^+) and $-\Delta H$. If a

correction were made in this graph for the difference in origin of the separate bicarbonate experiments (figure 5) the agreement would be considerably better.

Whether or not r remains constant for different intracellular hydrogen-ion concentrations cannot, as has been said, be determined from the data. Any variation in r with (H^+) would result in the (horizontal) displacement of the points in figure 7 to greater or lesser values of (H^+), thus changing the shape of the curve. For this reason the approximately rectilinear nature of this curve has no significance.

Though the results obtained on one atrium only will be described here, it may be remarked that a number of atria have been examined in this manner. The fixed anion term, α, appears to vary between 2.2 and 2.8 millimols per liter; the rate of change of $-\Delta H$ with (H^+) varies considerably, some atria being nearly twice as sensitive, and others only half as sensitive as the one described here. The general relations with respect to distribution of bicarbonate ion which have been described are consistently carried out in each of these tissues, however.

The fact that the contractility of the muscle is progressively decreased as the intracellular acidity is increased (a fact shown by any one experiment with increasing concentrations of carbonic acid, without examination of several bicarbonate solutions) is interesting in relation to the physiology of the contractile process. A similar result was obtained by Meyerhof (1922), who found that valeric acid prevented both contraction and the formation of lactic acid in the skeletal muscle of the frog. The observation precludes the suggestion, sometimes made, that the liberation of hydrogen ions (from lactic acid) is the immediate cause of the development of increased tension in the muscle.

The action of other acids on atrial tissue. The progressive loss of contractility with increasing intracellular acidity makes it possible to examine directly the penetration of other acid substances, either ions or molecules, into atrial tissue. A group of experiments on one atrium with HCl, NaH_2PO_4, lactic, acetic, propionic, *iso*-butyric and *n*-valeric acids are illustrated in figure 8. The penetration of the hydrogen ion was tested by adding to carbonate-free Ringer's solution sufficient HCl to increase the acidity from pH 8.0 to pH 6.0. For testing the penetration of the other acids, a Ringer's solution was prepared with the sodium salt and adjusted to pH 8.0. After the atrium had been exposed to this solution for some time and the contractility had become constant, a sufficient quantity of the acid (NaH_2PO_4, lactic acid, etc.) was added to bring the reaction to pH 6.0.

The effects of NaH_2PO_4, lactic acid and acetic acid are practically identical with those of HCl; in comparison with the rapid penetration of CO_2, the penetrating power of the hydrogen ion, the primary phosphate ion and lactic acid is almost negligible. Propionic, buytric and valeric

acids cause a loss of contractility at relatively increasing speeds, indicating increasing penetrating power with increasing molecular size. But even the most rapid of these, valeric acid, falls far behind CO_2; equilibrium, as indicated by the attainment of a constant amplitude of contraction, is not reached for 40 to 60 minutes, as compared with 3 to 5 minutes for CO_2.

From these facts it is concluded that atrial tissue is relatively impermeable to the hydrogen ion, the primary phosphate ion and lactic acid; and but slightly permeable to the molecules of acetic, propionic, butyric and valeric acids. Since the concentrations of hydrogen and hydroxyl ions in aqueous solutions are rigidly related, a condition of impermeability to the hydrogen ion indicates a corresponding impermeability to the hydroxyl ion.

The penetration of non-acid anions. It has been shown that the atrial tissue is apparently freely permeable to bicarbonate ions, but relatively impermeable to hydrogen (or hydroxyl) ions and the secondary phosphate ion. The question arises whether the tissue is permeable to anions which do not have acid properties, such as the lactate, the acetate or the secondary phosphate ion. It is obvious that the penetration of such ions would not increase the intracellular acidity and consequently would not reduce the contractility. Therefore the possible penetration of these ions cannot be tested directly.

The only instance in which the acid penetrates with sufficient rapidity to permit the observation of the action of the acid in the presence of various concentrations of the anion is valeric acid. But even in this case it is apparent that true equilibrium conditions are reached with difficulty, and the experimental results have little more than qualitative significance. For this reason detailed data will be omitted. In experiments with valeric acid and sodium valerate the atrium was left in each sodium valerate solution for one hour, and from 40 to 90 minutes were allowed for equilibrium after the valeric acid was added. The results are sufficient to show that the action of valeric acid is modified by the concentration of sodium valerate in the fluid, just as the action of CO_2 is modified by the concentration of bicarbonate, and in the same direction and to approximately the same degree. These results may therefore be considered to be qualitative evidence of the penetration of valerate ion.

Experiments with mixtures of carbonic acid and other acids. The relations which have been described above in respect to the penetration of bicarbonate ion (equation 8) are entirely similar to those obtaining in marine eggs (Smith, 1925). Either because of differences in inherent structure or because of their smaller size, these eggs proved to be readily permeable to acetic and propionic acids. They were likewise "permeable" to acetate and propionate ions, and it was shown that α had the same value

in carbonate, acetate and propionate solutions. By examining the effects of the three acids separately, it was possible to prove what has been assumed for atrial tissue in proposition b; namely, that the observed effects are attributable to the intracellular liberation of hydrogen ions, because the concentrations of these three acids (carbonic, acetic and propionic) required to inhibit cell division are related inversely as their respective dissociation constants. With a view to examining the significance of the anion term, α, a number of experiments were performed with the eggs using mixtures of carbonates and acetates, or of carbonates and propionates. It was found that in such mixtures, α, was distributed between the two anions in proportion to the product of the concentrations and the dissociation constants of the respective acids. The following equation was developed and tested for this distribution:

$$\alpha_{A_1} = \frac{\alpha}{1 + \frac{(HA_2)k_2}{(HA_1)k_1}} \tag{11}$$

where α has the value of the fixed anion term when only one acid is present.

The net result of this distribution of α between the two anions is that the action of one acid is modified by the presence of a second. (Were it not for α, the presence of the second acid would not modify the action of the first, since any two acids in equilibrium with the same hydrogen-ion concentration in the fluid would establish the same hydrogen-ion concentration in the cell, providing the cell-fluid distribution were the same in respect to HA and A^- for both acids.) This modification of the action of one acid by the presence of a second acid in the cell makes it possible to test the penetration of any weak-acid anion, even when the cell is impermeable to the corresponding acid. Suppose, for example, that the atrial tissue is permeable to CO_2, the bicarbonate ion and the lactate ion, but impermeable to lactic acid. The lactate ion must reassociate in the tissue with hydrogen ions liberated from carbonic acid, and thus form lactic acid; more CO_2 and more lactate ion will pass from fluid to cell to form more lactic acid, and this cycle will continue until, in the cell, carbonic acid and the bicarbonate ion on the one hand and lactic acid and the lactate ion on the other are in equilibrium with the same hydrogen-ion concentration; which is precisely the same equilibrium that would be established if lactic acid had migrated directly from fluid to cell. Consequently the addition of any penetrating anion to carbonate mixtures should modify the action of CO_2 in the same manner as it would if the acid could also penetrate.

On the assumption that the term α has the same significance in the

atrial tissue and in the marine eggs, equation 10 may be written for mixtures of two weak acids,

$$(12) \qquad (H^+) = \frac{[HA_1]k_1}{\dfrac{r\alpha}{1 + \dfrac{[HA_2]k_2}{[HA_1]k_1}} + r[A_1^-]}$$

This equation makes it possible to predict that the addition of a second penetrating acid, or of a penetrating anion, will increase the effectiveness of CO_2, and by taking r equal to unity and rearranging the equation, the exact degree in terms of $-\Delta H$ to which the action of CO_2 will be modified can be stated.

Experiments illustrating the action of CO_2 in the presence of definite bicarbonate-ion concentrations when lactate, phosphate, acetate, propionate, butyrate and valerate were added to the solution are given in figure 9. In each case one hour elapsed between the immersion of the tissue in a particular mixture and the CO_2 determination. Lactate, phosphate (NaH_2PO_4 + $NaHPO_4$) and acetate do not appreciably modify the action of CO_2. Propionate and butyrate modify the action of CO_2 somewhat, and in the right direction, while valerate modifies the action of CO_2 to precisely the extent to be predicted from (12). The solid line (valerate-carbonate mixture) in figure 8 is drawn with a slope calculated with the aid of (12) and based on that of carbonate alone (dotted line). A value of 2.8 millimols per liter was arbitrarily used for α.

It is concluded from such experiments as these that the atrial tissue is impermeable to the lactate ion, the secondary phosphate ion and the acetate ion; it is slightly permeable to the propionate and butyrate ions, and sufficiently permeable to the valerate ion to allow a condition of apparent equilibrium to be reached in one hour.

To test further this conclusion relative to the valerate ion, a series of experiments was performed with bicarbonate solutions to which varying quantities of valerate had been added; a series of such experiments from one atrium is given in figure 10. In this case the tissue was first tested with CO_2 at several bicarbonate concentrations (dotted lines) to determine the value α, and then tested in the valerate-bicarbonate mixture, allowing one hour for equilibrium with each new mixture. The solid lines were drawn with slopes calculated from the bicarbonate curves and placed to encompass the majority of data without regard for point of origin. At moderate concentrations of valerate, the agreement with the calculated values is good, but at larger valerate concentrations the contractility is depressed to an excessive degree. At these higher concentrations the reduction in contractility is poorly reversible, and it is possible that the problem becomes complicated under these conditions by a specific action of valeric

acid, analogous to the specific action of benzoic and salicylic acid on the egg cell (Smith, 1925).

The action of HCl on atrial tissue. It has been pointed out in discussing the experiments illustrated in figure 8 that when HCl is added to $NaHCO_3$-free Ringer's solution to bring the acidity to pH 6.0, there results a slow but progressive loss of contractility. An equilibrium level is not reached except after relatively long periods of time (one to several hours). If, in successive experiments, progressively larger quantities of HCl are added, the rate of loss of contractility becomes greater and greater until at pH 2.8 to 3.5 the contractility is reduced with great speed, and may be completely abolished by exposure of a few minutes.

This action of HCl in decreasing the contractility of atrial tissue is surprisingly reversible; when returned to a neutral or slightly alkaline (pH 7.0 to 8.0) $NaHCO_3$-free Ringer's solution the contractility returns, much more slowly than in the case of CO_2, to a large fraction of its initial value. The addition of a small quantity of $NaHCO_3$ will rapidly restore the contractility to its full value, after which the $NaHCO_3$ can be removed by washing with $NaHCO_3$-free Ringer's solution. In this manner the contractility of a particular atrium can be rapidly abolished time after time by HCl, without apparent injury to the tissue (though long exposures to very acid solutions, pH 3.0 to 4.0, are definitely injurious). In fact the contractility of an atrium which has been in use with CO_2 for some time may be greatly improved by an HCl treatment just sufficient to abolish contractility.

The qualitative aspects of this HCl action suggest that the tissue is permeable to HCl, or the hydrogen ion, but that the permeability is restricted or conditioned in such a manner that a large concentration of hydrogen ions is required in the fluid to force them into the cell; and that the rate of movement of hydrogen ions between fluid and cell is relatively slow. But since, at the constant level which is reached in solutions of pH 5.0 to 6.0, the loss of contractility is small compared with that in CO_2 solutions, it must be recognized that the final or equilibrium distribution of hydrogen ions between fluid and cell is not one of equality, but one in which the concentration outside is considerably greater than the concentration inside the cell.

The apparent "restricted" permeability to HCl is of such theoretical interest that a number of experiments were performed with HCl, lactic acid and disodium phosphate. The long periods of time required to reach an equilibrium level preclude a quantitative study such as was made of the action of CO_2, so it was only possible to determine the rate at which contractility is lost in various solutions. This process is uniformly linear throughout the early part of the exposure (10 to 40 minutes) and can be expresed by the loss of contractility (in centimeters of the isotonic myogram) per contraction (or per unit of time): dH/dc.

A number of experiments with HCl, lactate and phosphate solutions are illustrated in figures 11, 12 and 13, in which the atria were contracting spontaneously at a frequency of about 36 per minute. (These experiments were performed before the denoded atrium was prepared, and in Ringer's solution with no $MgCl_2$, formula I.) In these figures the dots, circles, etc. represent different atria tested at various hydrogen-ion concentrations.

The rate of loss of contractility, dH/dc, is a logarithmic function of pH, and it follows from the 2:1 slope of the curve that dH/dc is a function of

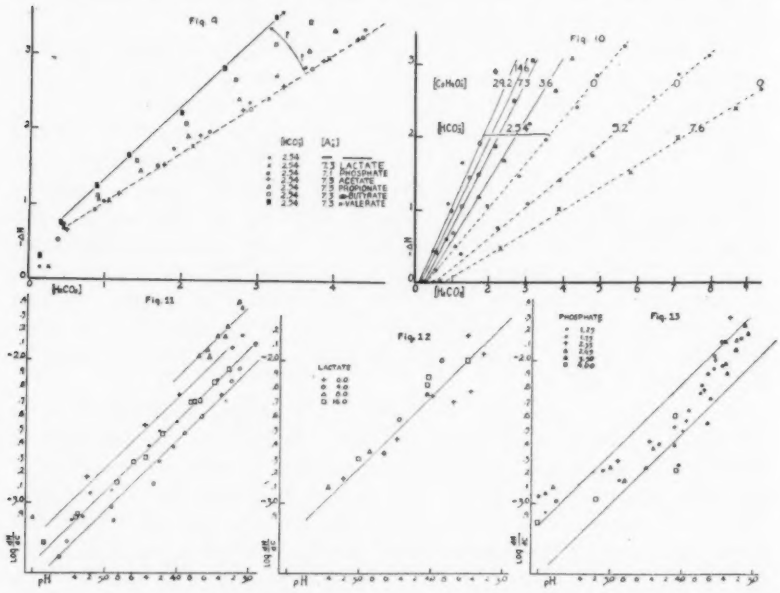


Figure 9 is described in the text on p. 436; figure 10, p. 436; figures 11, 12, and 13, p. 438

$\sqrt{[H^+]}$. This fact accords with the interpretation that dH/dc is an expression of the rate of penetration of the hydrogen ion. The effects of HCl, phosphate and lactate are practically identical, differing no more than do individual atria in HCl solutions. We may, therefore, believe that the effects of phosphate and lactate solutions are attributable solely to the hydrogen ion.

It is concluded from these experiments that the hydrogen ion (perhaps with chlorine or other anions) will penetrate the atrial tissue, and that the equilibrium condition is characterized by a large concentration difference between fluid and cell.

Other evidence bearing on this conclusion may be mentioned here. Cell division in *Asterias* and *Arbacia* eggs (Smith and Clowes, 1924a and b) and *Echinarachnius* eggs (Smith, 1925) is repressed by CO_2 at intracellular hydrogen-ion concentrations differing not very greatly from pH 6.5; in the absence of a penetrating acid (HCl and phosphate solutions) cell division in these eggs is repressed at extracellular hydrogen-ion concentrations of pH 4.6 to pH 4.8. It has been suggested regarding the action of HCl, that "above a certain concentration of hydrogen ions the semipermeable properties of the cell may be so modified that the hydrogen ion migrates from fluid to cell; or it might be supposed that HCl inhibition is an indirect consequence of some action on the (cell) cortex. Lastly, the possibility remains that there is, at all hydrogen-ion concentrations, an equilibrium between cell and fluid with respect to the hydrogen ion which involves a concentration gradient of such an order that an external hydrogen-ion concentration of pH 4.6 is required to establish the critical acidity in the cell" (Smith, 1925, p. 365). In the light of the above evidence, the last hypothesis appears to be correct. The differences in the action of non-penetrating acids (HCl, lactic acid, etc.) and penetrating acids (CO_2, etc.) are attributable to differences in the equilibrium distribution of hydrogen ions and of penetrating molecules. These differences in action have been shown on many tissues, of which the following may be mentioned: Overton (1902), *Spirogyra:* Bethe (1909), *Medusae:* Harvey (1911), *Paramoecium, Spirogyra, Elodea:* Warburg (1910) and Loeb and Wasteneys (1915), marine eggs; Crozier (1916), *Chromodoris zebra:* Haas (1916), plant tissues; Collett (1919, 1920a), *Paramoecium:* Jacobs (1920), *Symphytum peregrinum* and protozoa; Loeb, J., (1922), *Fundulus* eggs; Loeb, L. and Gilman (1924) amoebocyte cultures. Gustafson (1920) found that the respiration of *Penicillium chrysogenum* was not affected by increasing the acidity of phosphate and sulphate solutions from pH 8.0 to 4.0. MacArthur (1920) found that planarians tolerate an acidity of pH 4.7 to 4.9. Lewis and Felton (1921) report that tissue culture cells exhibit considerable growth at pH 5.5 and will resist much more acid solutions for short periods. Fischer (1921) states that fibroblast cultures may grow for 4 to 6 generations in plasma at pH 5.5. Dale (1915) found that a glycocoll solution of pH 4.0 was required to kill *Paramoecium* in the same time as a sodium acetate (!) solution of pH 5.5. Krontovski and Rodzimovska (1922) found that a lactate solution of pH 4.0 was required to kill lymphocytes, spleen cells, reticular cells and fibroblasts in the same time as an acetate (!) solution of pH 5.3.

It appears that in the absence of penetrating acids (such as CO_2, acetic acid, etc.) an acidity of pH 4.5 to 5.0 is tolerated for a considerable time by most tissues, while the presence of such penetrating acids suffices to suspend vital activities in much less acid solutions.

Discussion. The results obtained with atrial tissue are summarized in table 3, together with data on marine eggs which have been reported previously. The penetration or non-penetration of anions is indicated by plus or minus signs. The term "direct" indicates that the evidence of penetration or non-penetration of ions is obtained by direct qualitative action on vital activities, in contradistinction to evidence which involves differences in quantitative relations.

It will be observed that, for both atrial tissue and the marine eggs, an anion penetrates only when there is present a corresponding acid molecule which is able to penetrate. This fact excludes the possibility that these tissues are freely permeable to anions in general and raises the question

why the penetration of anions is dependent on the penetrating power of the corresponding acid. The possibility exists, of course, that the intracellular concentration of these anions is established by direct migration between fluid and cell; but such an explanation makes it necessary to attribute the penetration or non-penetration of anions to differences in their physical properties—an hypothesis which appears to be extremely improbable. The fact that the physical properties of *molecules* play an important part in determining their penetrating power is a direct consequence of the heterogeneity of protoplasm, and of the fact that molecules

TABLE 3

Penetration of molecules and ions into Echinarachnius eggs and turtle atrium

Echinarachnius eggs

	MOLECULE	ION	EVIDENCE
CO_2	+	+	Direct
Acetic	+	+	Direct
Propionic	+	+	Direct
Lactic	−	−	
$H_2PO^-_4$	Absent	−	Direct
$HPO^=$	Absent	−	
$HC_4H_4O^-_6$	Absent	−	Direct
$C_4H_4O^=_6$	Absent	−	
OH^-	Absent	−	Direct
H^+	Absent	−	Direct
Atrium			
CO_2	+	+	Direct
Valeric	+	+	Direct
Butyric	±	±	
Propionic	±	±	
Acetic	−	−	
Lactic	−	−	
$H_2PO^-_4$	Absent	−	Direct
$HPO^=_4$	Absent	−	
OH^-	Absent	−	Direct
H^+	Absent	−	Direct

will distribute themselves freely and independently in the various parts and components of heterogeneous systems according to their physical properties. Ions, on the other hand, must either migrate in cation-anion pairs, or by the exchange of one anion for another through an intermediary combination with some cation; in either case the status of an ion at any instant is the status of a salt (ionized cation-anion pair). Since the physical properties of the salts of acids are not related to the physical properties of the respective acid molecules, there is no reason to believe that the latter could possibly determine the behavior of salts in either homogeneous or heterogeneous systems.

The interpretation previously accorded the data on the marine eggs seems preferable; namely, that an "equilibrium concentration of anion is established in the cell subsequent to, and in consequence of the entrance into the cell of the respective acid molecule, by some mechanism which operates on other components of the cell-fluid system."

Concerning this mechanism, we may develop a provisional scheme, without claiming for it the least degree of probability in its present form, merely for the purpose of seeing whether it is possible for any arrangement to account for the facts which have been observed.

It is recognized that molecules of suitable physical properties (such as those of a penetrating acid) enter the cell by a route which may be designated as "physical," since the penetration of such molecules is in no way dependent on chemical combination with constituents of the cell.

In addition to, and independent of this physical route, we may suppose that the cell is enveloped by a membrane which contains an intercalated system of "carrier" groups capable of combining with H^+ and Cl^- ion pairs (as, for example, $RNH_2 \cdot HCl$), and capable of transporting these ion pairs, and of establishing an equilibrium with respect to them, between fluid and cell; and we may further suppose that, solely as a consequence of the inherent properties of this membrane, the equilibrium distribution of hydrogen ions across it—i.e., between fluid and cell—may be unequal. (It is not necessary to consider at this time whether or not this unequal distribution involves work on the part of the membrane. It may be considered provisionally to be a consequence of a membrane hydrolysis, or of differences between the internal and external surfaces of the membrane in respect to rates of combination with, or affinities for H^+ and Cl^- ion pairs, or of any other property strictly inherent in the membrane.)

Though no attempt will be made to rigidly predict the equilibrium conditions of this system, we may inquire how far the observed phenomena are qualitatively in agreement with those consequences which might logically be expected from it.

In the absence of a penetrating acid, any change in hydrogen-ion concentration in the fluid would result (through the transportation across the membrane of H^+ and Cl^-) in a change in the hydrogen-ion concentration in the cell in the same direction, but to a greater or lesser degree dependent on the particular properties of the membrane. As a special case, and to fit the case in hand, we may imagine these properties to be such that to produce a given change in hydrogen-ion concentration in the cell there would be required a hundred-fold change in hydrogen-ion concentration in the fluid; and, at equilibrium, the hydrogen-ion concentration in the fluid would exceed that in the cell by a correspondingly large degree.

The same equilibrium condition would, we should expect, be reached

from changes on either side of the membrane. If, instead of adding HCl to the fluid, we increase the intracellular acidity by any means, the transportation of H^+ and Cl^- would tend to occur in the reverse direction. Should the increased intracellular acidity be a consequence of the penetration of CO_2, for example, the outward movement of H^+ and Cl^- (H^+ derived from carbonic acid and Cl^- from cell chlorides) would leave bicarbonate ions and free base to combine in the cell. Thus the appearance of bicarbonate in the cell would be a consequence of the penetration and intracellular dissociation of carbonic acid and of the transportation outward of H^+ and Cl^-, while no actual migration of bicarbonate ions between fluid and cell would have occurred. The same series of events would follow the penetration of any acid into the cell, and apparently the cell would be "permeable" to the anion of any penetrating acid. The action of HCl on marine eggs and on atrial tissue, and the apparent penetration of the anions of penetrating acids are qualitatively accounted for in this manner.

The conditions obtaining in the above system when at equlibrium depend, by definition, on the particular properties of the membrane and on the intracellular and extracellular hydrogen-ion concentrations. It is apparent that in the same cell, the same equilibria would not necessarily be reached in HCl solutions and in solutions of a penetrating acid and its salt. In the former case, the intracellular hydrogen-ion concentration is determined *solely by the extracellular hydrogen-ion concentration* (across the membrane), while in the latter case the intracellular hydrogen-ion concentration is determined jointly by the extracellular hydrogen-ion concentration (across the membrane) and by the *molecules of penetrating acid*. Therefore several possible circumstances must be recognized, of which four will be considered here: 1, when HCl but no penetrating acid is present in the fluid; 2, when a penetrating acid but no salt of that acid is present in the fluid; 3, when a penetrating acid and its salt are present in the fluid; and 4, when two penetrating acids and their salts are present in the fluid.

When HCl but no penetrating acid is present in the fluid the intracellular hydrogen-ion concentration will be determined, as has already been stated, by the extracellular hydrogen-ion concentration in accordance with the particular properties of the membrane. Without definite knowledge of these properties, the equilibria reached cannot, of course, be predicted. In the case of *Echinarachnius* eggs, it has been shown that in HCl solutions, and in solutions of non-penetrating acids (tartrates, phosphates and lactates), an acidity of pH 4.6 is required to completely repress cell division. The same data show that to produce the first evidence of repression, and to reduce the division rate to 50 per cent of the normal, pH 5.1 and 4.9 are required. These conditions of initial, half and complete

repression of cell division correspond, according to calculations based on the action of penetrating acids, to intracellular hydrogen-ion concentrations of approximately pH 7.15, 6.75 and 6.50 (Smith, 1925). These figures are considered as indicative of the equilibria reached by the eggs, in respect to intracellular and extracellular hydrogen-ion concentration, when only HCl is present.

In the presence of a penetrating acid, it is apparent that the cell will first tend to become as acid as the fluid; but the outward movement of H^+ and Cl^-, with the consequent formation of the salt of that acid in the cell, will lower the intracellular acidity. At equilibrium the acid will be distributed equally between cell and fluid (equation 1) and a sufficient quantity of anion will exist in the cell to restore equilibrium with respect to the membrane. This quantity of anion appears in the previous equations in the term α, and has been designated as the "fixed" anion. This "fixed" anion appears, in terms of this scheme, to be a consequence of those same properties of the membrane whereby, in HCl solutions, the *difference* in concentration of hydrogen ions in cell and fluid is maintained.

In the absence of the salt of the penetrating acid,

$$[H^+] = [A^-] \tag{13}$$

and (10) and (13) become, by rearrangement of (2)

$$(H^+) = \frac{[H^+]^2}{r([H^+] + \alpha)} \tag{14}$$

Since no variables other than $[H^+]$ and (H^+) are present, it appears that (14) might also apply to HCl solutions. *In which case the hydrogen-ion concentration of HCl and of simple solutions of penetrating acids should be identical when they produce the same intracellular hydrogen-ion concentration.* This follows if we equate the second terms of (10) and (14), cancel the identical denominators and write $[H^+]^z_{HA} = [HA]k$.

$$[H^+]_{HCl} = [H^+]_{HA} \tag{15}$$

The figures for $[H^+]_{HCl}$, for tartrate, phosphate and lactate solutions corresponding to initial, 50 per cent and complete repression of cell division in *Echinarachnius* eggs have already been cited as pH 5.1, 4.9 and 4.6. The corresponding values of $[H^+]_{HA}$ (which equals $\sqrt{[HA]k}$) in carbonate, acetate and propionate solutions as determined experimentally by extrapolation of [HA]k to zero value of $[A^-]$, are pH 5.1, 4.9 and 4.8. The agreement is significant, in view of the fact that the action of penetrating acids in the presence of their salts is demonstrably a consequence of increased intracellular acidity (see p. 356, Smith, 1925), and there is no rea-

son to expect any relation between the action of simple solutions of penetrating acids and the action of HCl unless the latter is also attributable to increased intracellular acidity.

In the presence of a penetrating acid and its salt, the two components which determine the intracellular hydrogen-ion concentration, namely, $[H^+]$ and [HA]k, become independent by virtue of the introduction of A^- into the fluid, and these components now operate separately on the intracellular reaction. If (10) is rearranged, by substituting $[HA]k/[H^+]$ for $[A^-]$ and neglecting r, into the form

$$(H^+) = \frac{[H^+]}{1 + \frac{\alpha[H^+]}{[HA]k}} \tag{16}$$

it is seen that (H^+) is determined by the ratio of [HA]k to $\alpha\,[H^+]$. According to the above scheme, these terms are interpreted as expressions of the relative *intracellular hydrogen-ion producing power* of molecules of penetrating acid on the one hand, and of the extracellular hydrogen-ion concentration, acting across the hypothecated membrane, on the other.

In the presence of two penetrating acids and their salts, the intracellular hydrogen-ion concentration becomes dependent on the three independent components, $[H^+]$, $[HA_1]k_1$ and $[HA_2]k_2$. Taking equation 16 as expressing most clearly the idea involved, it is apparent that the term [HA]k, which has been interpreted as expressing the hydrogen-ion producing power of the penetrating acid, will be altered in the presence of two penetrating acids. Since the hydrogen-ion producing power of two penetrating acids is proportional to the sum $[HA_1]k_1 + [HA_2]k_2$, we may substitute this sum for [HA]k and (16) becomes

$$(H^+) = \frac{[H^+]}{1 + \frac{\alpha[H^+]}{[HA_1]k_1 + [HA_2]k_2}} \tag{17}$$

On rearrangement this becomes the previously recorded equation (12). This equation was shown to hold true in mixtures of two penetrating acids on marine eggs, and it was substantially indicated by similar experiments on atrial tissue.

In résumé, the above scheme can account for the action of HCl, and for the apparent penetration of anions of penetrating acids, without invoking specific penetrating properties for various anions; without attempt at rigid prediction, it explains the appearance of "fixed" anion in the cell (equation 10) and the distribution of the fixed-anion term, α, between the anions of two penetrating acids when two such acids are present in the fluid (equation 12).

In its present form, however, equations 10 and 14 are obviously irra-

tional, because they lead to excessively alkaline intracellular reactions when the cell is placed in neutral solutions. The inaccuracy unquestionably resides in the term α, which has been considered to be a constant on the basis of the experimental data. It seems probable that α is not a constant but a variable which is dependent on other components ((H^+) or $[H^+]$, etc.) of the system, and which approximates constancy under the experimental conditions. It is possible that at less acid intracellular reactions than those examined, α may become a very small or even a negative value, thus rendering the cell as acid or more acid than the fluid.

Although the transportation mechanism described above has been spoken of as involving only H^+ and Cl^- ion pairs, it is apparent from the nature of the postulated mechanism that any acid which might be present could be transported in the same manner. But where HA is a non-penetrating acid its effects would be identical with those of HCl, and no A^- would tend to move across the membrane in either direction except in consequence of a change in $[H^+]$ or (H^+), and then only in proportion to the relative quantities of Cl^- and A^- combined with the "carriers."

SUMMARY AND CONCLUSIONS

A preparation is devised from the excised atrial tissue of the turtle which is suitable for the examination of the action of carbonic and other acids on muscular contraction.

It is concluded from physiological methods that this tissue is freely permeable to CO_2 and apparently permeable to the bicarbonate ion. Under the observed conditions the bicarbonate-ion concentration in the cell appears to be some fraction (which is probably near unity) of the sum of the bicarbonate-ion concentration in the fluid and a constant, or approximately constant quantity.

Atrial tissue is relatively impermeable to the primary and secondary phosphate ions, to lactic acid and the lactate ion, and to acetic acid and the acetate ion; it is slowly permeable to valeric acid and apparently permeable to the valerate ion; propionic and butyric acids, and the propionate and butyrate ions occupy intermediate positions between valeric and acetic acids.

From the action of HCl, lactates and phosphates, it is concluded that the hydrogen ion penetrates atrial tissue providing a sufficient concentration difference between fluid and cell is established; and that the final equilibrium is characterized by a considerable difference in concentration of hydrogen ions in fluid and cell.

From the above facts, and from similar facts relative to marine eggs described in a previous paper, it is concluded that the apparent penetration of certain anions is not a consequence of migration of these anions

from fluid to cell. In view of the action of HCl, it is provisionally suggested that an intracellular concentration of anion is established by a redistribution of H^+ and Cl^- ion pairs between cell and fluid subsequent to and in consequence of the penetration of the respective acid molecule.

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STUDIES OF THE NERVE IMPULSE

II. The Question of Decrement[1]

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Received for publication December 28, 1925

In a previous paper two of us (Davis and Brunswick, 1926) have described a method for measuring the action currents of excised mammalian nerve by means of a string galvanometer. By the calculation there described, we obtain a value proportional to the average electromotive force developed by the nerve fibers, and reasons are there set forth for believing this figure to be a valid relative measure of the intensity of the nerve impulse. Due to certain insurmountable technical difficulties, chiefly that of determining what fraction of the total current passes through the galvanometer with a given combination of nerve, protecting fascia, lead-off electrodes, etc., it is impossible accurately to compare values obtained at one point with those from another point; but significant comparisons may be made of the intensities at a given point under various experimental conditions.

In the present paper we have applied this technique to a direct investigation of the question of conduction with a decrement in a region of nerve narcotized by alcohol vapor.

The conception of conduction with a decrement proposed by Szpilman and Luchsinger (1881) and developed by Dendrinos (1902), Fröhlich (1904), Lucas (1913) and others is too familiar to require detailed exposition. In brief the theory states that the nerve impulse in traversing a region exposed to the action of a narcotic undergoes a progressive diminution or decrement in intensity, and if the length be sufficient, is eventually extinguished. The depth of the narcosis determines the rate of the diminution. The laws of this diminution were never clearly stated, no satisfactory data for their formulation being available, although Lucas and Adrian in their diagrams assume (for simplicity) that it is linear (cf. Lucas 1913, p. 476, footnote), while a figure published by Verworn (1914) implies a logarithmic law.

Probably less familiar is the recent work of Kato (1924) in Japan, reports of which first reached our hands after the completion of most of the

[1] Preliminary report: Davis, Forbes, Brunswick and Hopkins (1925).

experiments described in this paper. Kato doubted the existence of decremental conduction and undertook to examine critically the evidence upon which the theory was based. His doubts arose from the fundamental dissimilarity between the all-or-none behavior of normal nerve and the decremental behavior supposed to be induced by narcotics. The all-or-none relationship shows that the energy of the impulse must be supplied locally by the nerve fiber; and we should naturally suppose, as Kato points out, that the same would be true in narcosis, the impulse being transmitted at a lower *but constant* intensity if at all.

Our attention was called to the question by a contradiction, pointed out by Forbes, Ray and Griffith (1923), between certain conclusions of Adrian (1914b) and the implications of the membrane theory of protoplasmic conduction (cf. Lillie, 1923, and earlier workers there cited). Adrian observed that the velocity of conduction is constant in various lengths of equally narcotized nerve. Assuming decremental conduction in a region of narcosis, he concluded that the velocity of conduction was independent of the magnitude of the impulse. The membrane theory, on the other hand, demands a definite relationship; namely, that the velocity be proportional to the magnitude and the rate of development of the electrical potential difference associated with the active state. Forbes, Ray and Griffith (1923) showed that the predicted correlation between intensity (as judged by the action current) and velocity exists when the impulse is traveling in the relative refractory period of a previous impulse; and the same conclusion may be drawn from the more accurate data of Gasser and Erlanger (1925). We were thus led to the hypothesis that the impulse, as judged by the electric response, might be uniformly depressed in a region of narcosis as suggested by Adrian's findings on the velocity, and not show the decrement usually assumed; and the present experiments were designed to test this hypothesis.

It is worth while to point out the indirect nature of the evidence for the theory of conduction with a decrement, and to review briefly the critical examination of this evidence by Kato and his collaborators. One fundamental type of observation is that if stimuli of a given strength are applied at various points in a narcotized region of nerve, response fails first for the longest length of narcotized nerve and last for the shortest. After response has failed from a given point it may be evoked once more by increasing the strength of the stimulus. The obvious criticism that the stimulating current may be "spreading" along the nerve and stimulating outside the narcotizing chamber is met by crushing or ligating the nerve just below the stimulating electrodes, which effectively stops the response to the "inside" stimulus (cf. Lucas, 1913). Kato, however, shows that crushing the nerve hinders the spread of stimulus, and is therefore not a proper control. By careful measurements of the latent period of maximal

muscular contractions with different strengths of stimuli he shows quite convincingly that it is possible for the stimulus to take effect as far as 40 mm. from the electrodes, and that spread must be considered even with just maximal shocks. Various ingenious tests support this proposition qualitatively, and it is further shown that failure of conduction is independent of the length of narcotized nerve to be traversed when mechanical or thermal stimuli are employed. There is no necessity to assume that a stronger nerve impulse is evoked by a stronger stimulus (cf. Lodholz, 1913; Rehorn, 1918; Adrian and Forbes, 1922). The present author whose work with Adrian is here referred to, now believes the experiments in question to be valueless because of lack of proper control of current spread, and the conclusion drawn from them unjustified.

Another line of evidence in favor of decrement is the familiar observation that conduction is suspended sooner in a long stretch of nerve than in a short one, when both are subjected to equal strengths of narcotic. The stimuli, in this case, are applied above the narcotized regions. This experiment is usually performed with small narcotizing chambers only a few millimeters across. Kato, repeating the experiments with longer nerves, found that the classical observations may be verified provided the smaller chamber is less than 6 mm. long, but otherwise conduction fails across both long and short stretches of narcotized nerve at the same time, within limits of experimental error. For lengths of more than 6 mm. therefore, evidence in favor of decrement is lacking. Kato interprets the anomalous result with short lengths as due to a diffusion gradient of the narcotic along the nerve for a few millimeters inside the chamber. Unfortunately no direct evidence is brought forward to support this view, and we shall show later that another interpretation is possible; but this in no way invalidates Kato's observations and conclusions for the greater lengths of nerve.

Lucas in a careful study of the effect of alcohol on amphibian nerve (1913) measures the least interval between stimuli which will give a summated muscular contraction. Under the influence of alcohol this interval is increased. This might be due either to slower recovery or to decremental conduction. In the latter case a second impulse might be initiated as early as ever, but, being subnormal in magnitude, fail to pass the region of decrement. The rate of recovery is measured by a method which need not be detailed, and is found to be unchanged. However, the strengths of stimuli in terms of which recovery is measured, are always expressed, not in absolute units, but in terms of the threshold. Now under the influence of alcohol the threshold rises. It may be true that the *relative* recovery so measured is not prolonged, but obviously the time which must elapse after a previous response before a given stimulus will cause excitation is definitely increased. Presumably therefore there will be a prolongation of

the time which must elapse before the weakest impulse capable of propagation can follow a previous impulse. Lucas's observations thus may be explained without invoking the theory of decremental conduction. In another paper (Adrian and Lucas, 1912), the prolongation of the least interval is correlated with the length of nerve narcotized. This would seem to prove decremental conduction, but Kato, repeating the experiment, finds no correlation. The reason for this difference is not readily apparent, unless we assume considerable spread of stimulus in Adrian and Lucas' experiment.

The size of the electric response of the nerve was used as a measure of intensity of the nerve impulse by Boruttau and Fröhlich (1904), and the presence of decrement apparently demonstrated by the progressive diminution in the action current from point to point along the narcotized region. Kato and Lucas both call attention to the obvious fallacy in this experiment; namely, the failure to prove that no fibers were blocked completely by the narcotic between the two electrodes in the chamber. If this occurs it is obvious that the response at the second electrode will be diminished to a greater degree (relatively and absolutely) than that at the first electrode. Forbes and Ray (1923) offer somewhat similar evidence, showing that either half of a long nerve will conduct an impulse, but that an impulse will not traverse the entire length. They offer no proof that the same fibers were tested in each case, and the result is easily explained if we imagine every fiber blocked at some point along its course but not all at the same point.

Kubo, one of Kato's collaborators, guards against such fallacies by recording the action current from a third point on the nerve beyond the narcotizing chamber. Only while the response here remains undiminished, is it certain that no fibers are being totally blocked in the chamber. The experiment is only valid, therefore, while the response at this control electrode remains constant. During this period of constancy Kubo obtains a marked depression of response within the chamber, and to an equal degree at both of his "inside" electrodes. From this he concludes that the impulse (for which he tacitly assumes the height of the action-current record to be a measure) suffers no decrement in a narcotized region.

Kubo's control is not quite perfect, however, even though a basis in fact for the following criticism is highly improbable. Kubo employs the sciatic nerve of the toad, which gives off a number of branches. He makes no specific mention of the size of these branches or their position relative to his electrodes, but he does mention that at the beginning of the experiment the "action currents. . . . . at the three points . . . . were equal in most of the experiments." Since he is recording monophasically, using the crushed distal end of the nerve as his indifferent electrode, there is a varying length of nerve in his recording circuit, and consequently a varying resist-

ance. We should therefore expect the normal action current from the first electrode to be the smallest, and that from the control to be the largest. That they are equal suggests a greater number of active fibers at the first electrode and fewer at the control. If this is the case, some of the fibers which contribute to the responses from the narcotized region would be uncontrolled. If we suppose that these fibers, possibly due to a more exposed position in the nerve trunk or to a greater susceptibility, or to the trauma of cutting the branches, cease responding one after another while the responses of the controlled fibers are only partially depressed, we might explain Kubo's results without abandoning the idea of decrement. Since the first electrode has (presumably) a larger proportion of these uncontrolled fibers, the total response there might be reduced, say 25 per cent, by their ceasing to function, while the response of the (controlled) remainder might fall 10 per cent due to partial narcotization and decremental conduction. At the second lead there might be a 10 per cent reduction due to failure of uncontrolled fibers, and a 25 per cent reduction due to narcotization and decrement. The total reduction would be 35 per cent in each case. But we must admit the extreme improbability that such a balancing would occur continuously throughout the course of an experiment, and in numerous experiments without exception.

In our own experiments, which closely resemble those of Kubo, we believe that we have ruled out the possibility just described and furthermore have controlled possible changes in resistance of the nerve under the influence of narcotics, which Kubo does not mention. We employ an unbranched nerve, the peroneal of the cat, and test it for the presence of blocked and therefore uncontrolled fibers. It is worth noting that we frequently observe action currents of nearly identical height from the last and from the next to last electrode on a normal undamaged nerve, just as Kato did. Two of us have investigated this phenomenon (Davis and Brunswick, 1926) and interpret it as a depression of the response of each fiber due to the influence of the neighboring crush. Such depression does not invalidate the use of the last electrode as a control, since all fibers still contribute to the action current, so that Kubo's experiments are probably sounder than might appear from the foregoing criticism.

We may state in advance that our results on mammalian nerve with the additional precautions and controls just mentioned are in entire agreement with those of Kubo on amphibian nerve; and that quite independent of Kato we had come to the same conclusion as to the absence of decrement in such lengths of nerve as we have both investigated.

METHOD. The experiments to be described were all performed on the peroneal nerve of the cat. In a previous paper (Davis and Brunswick, 1926) we have given details of the technique of preparation which affords us ten to twelve centimeters of unbranched nerve, most of it uniformly

circular in cross section. The nerve is mounted in the moist chamber there described, supported by the chloride-coated silver electrodes. Monophasic action currents are recorded photographically by means of a string galvanometer whose sensitivity is carefully controlled. The excursions are measured under magnification, and an average of several excursions under identical conditions is calculated. The resistance of the nerve is frequently measured, and corrected to allow for certain galvanometer characteristics. Then, from resistance and deflection, the intensity of the action current is calculated in terms of electromotive force. Correction is made for "escape" of stimulating current. The proportion of locally damaged fibers is investigated by stimulating at various points while recording from the last control electrode (no. 5). In no experiment here considered did this test indicate more than 3.4 per cent of blocked fibers.

It is perhaps worth emphasizing one feature of the recording device; namely, that our string, being relatively slack and heavy, behaves very much like a ballistic galvanometer. That is, the height of its excursion is proportional (nearly) to the quantity of electricity flowing through it, irrespective of the time relations of the discharge. This holds true with considerable accuracy up to durations of 4σ, and within a few per cent to 5σ. We can therefore neglect changes in the time relations of the action current at any given point resulting from uneven narcosis of the nerve and consequent unequal slowing of the impulses in various groups of fibers. The argument is similar to that whereby we find (loc. cit.) that we may neglect the normal differences in rate of conduction shown by Gasser and Erlanger (1922). Even the possible coincidence of the narcotic exerting a preferential slowing effect on the fibers which normally conduct most slowly would cause no significant error since the relative proportion of such slow fibers in a mixed nerve like the peroneal is quite small (cf. Gasser and Erlanger, 1923).

In all of our experiments we employed the chamber previously described (Davis and Brunswick, 1926) and illustrated in figure 1. This gave us a narcotizing chamber 45 mm. in length, containing three lead-off electrodes spaced 15 mm. apart, with 7 mm. between each partition and the nearest electrodes. This inner chamber was closed by a separate glass lid and all openings between it and the stimulating and control chambers sealed with vaseline. Lead 4 was located in a space 13 mm. wide between the two hard-rubber partitions, sealed off without ventilation and serving as a trap to check possible leakage from the narcotizing chamber to the control chamber. Lead 4 served as a control lead, but in several experiments it was rendered useless by diffusion of narcotic down the nerve or leakage into its chamber. The other control, lead 5, and the indifferent electrode, 6, were in an end compartment which, like the stimulating compartment, was ventilated by a fairly rapid stream (100 cc. per min.) of moist air.

The narcotic employed was alcohol vapor. The alcohol-Ringer mixture with which Lucas (1913) and Adrian (1914b) obtained the most constant results was not employed because of the difficulty of interpreting the electrical effects when the nerve is immersed in an electrolyte. The stream of air passing through the inner chamber could be diverted so as to bubble through either distilled water or a mixture of 5 to 6 per cent alcohol in water. The bubbling tube extended about 1 cm. below the surface of the liquid, and the rate of flow was maintained at about 200 cc. per minute.

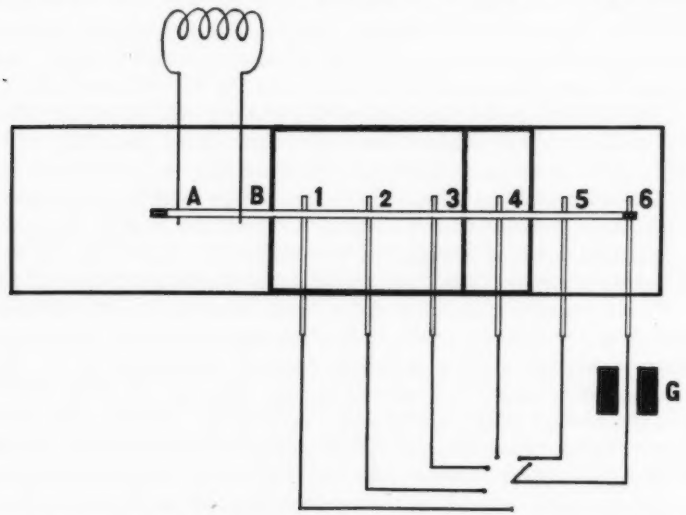

Fig. 1. Diagram of chamber and its connections. Distance between electrodes = 15 mm. Platinum stimulating electrodes at *A* and *B*. Silver lead-off electrodes at *1*, *2*, *3*, *4*, *5* and *6*. On the nerve, black indicates crushed areas. A thermometer is placed in the outer chamber near *A*. Pure moist air is passed through the outer chambers containing *A* and *B*, and *5* and *6*. Pure air or alcohol vapor may be passed through inner chamber containing *1*, *2* and *3*. Chamber containing *4* receives no ventilation.

No attempt was made to determine the concentration of the alcohol vapor, our object being merely to find empirically a strength which would slowly narcotize the nerve. The vapor-laden stream of air entered the nerve chamber through two openings on one side located respectively between leads 1 and 2, and 2 and 3, and escaped through three openings in the opposite wall directly beneath the respective leads. We assumed that this arrangement and the rapid rate of flow gave a uniform distribution of narcotic; but the point was not specifically tested, and certain features of the results suggest that it may not have been strictly true.

Our procedure, described in detail in the first paper of this series (loc. cit.), was to mount the nerve in the chamber and allow it to reach a steady state, frequently recording its action currents and measuring its resistance so that the control period would be strictly comparable to the test period. During this period the shift-of-stimulus test was also performed, and the strength of stimulus adjusted to about 20 per cent above maximal (for break shocks). The air current through the narcotizing chamber was then diverted to pass through the alcohol, and action currents were recorded every two or three minutes from each of the five electrodes until the response at the control leads had fallen perceptibly. Usually recovery was allowed, in order to detect possible changes in the response at the control lead. Such changes were almost never encountered when allowance was made for resistance changes. The resistance was measured frequently throughout the narcosis. As a rule it rose slightly with the admission of the alcohol vapor; but the change was not reversible, and we believe it to be due to slight drying of the nerve.

Results. The responses of the nerve during the period of narcosis are expressed as percentages of the average response recorded from the corresponding lead during the control period, after the steady state has been reached. The responses from each lead are plotted against time, as in figure 2. It is impossible to compare the absolute E.M.F. values from different leads because of probable differences in electrical leading-off conditions; and in any case comparisons of the relative depressions will show the presence or absence of decrement most clearly.

The results of a typical experiment are presented in figure 2. The response at lead 5, the main control, remains constant within experimental error for 15 minutes, showing that all fibers continue to conduct. At lead 4 the response is nearly constant, but there is evidence of some leakage of narcotic. Within the narcotizing chamber, however, as the alcohol vapor starts to flow the response begins to fall immediately and continues to fall progressively for the duration of the experiment, to a minimum of 68 to 70 per cent of normal. The significant feature is the parallel course of the depression at the three leads. At a given moment the relative depression is essentially equal at all three leads. There is no evidence whatever of conduction with a decrement. If decrement existed the depression of response should be greatest at lead 3 and least at lead 1. In this experiment the depression is identical at leads 1 and 2 (within experimental error), while at lead 3 it is slightly less. This divergence at lead 3 is probably to be explained either by *a*, unequal distribution of narcotic in the chamber; or *b*, too low a value for the normal at this point (only two control values having been obtained); or, more probably, *c*, less rapid penetration of the narcotic at this particular point.

The results of another experiment are presented in figure 3, confirming

those already described and extending the observations to a deeper stage of narcosis. Here we see the preliminary steady state, the slight scattering of the points above and below 100 per cent giving an indication of the range of experimental error. As before, the control at lead 5 remains constant for about 15 minutes. At lead 4 there is a moderate and progressive depression, probably due to leakage of alcohol vapor. Within the narcotized region, as before, the depression is essentially parallel at the three leads. The depressions of response at leads 1 and 3 are equal within

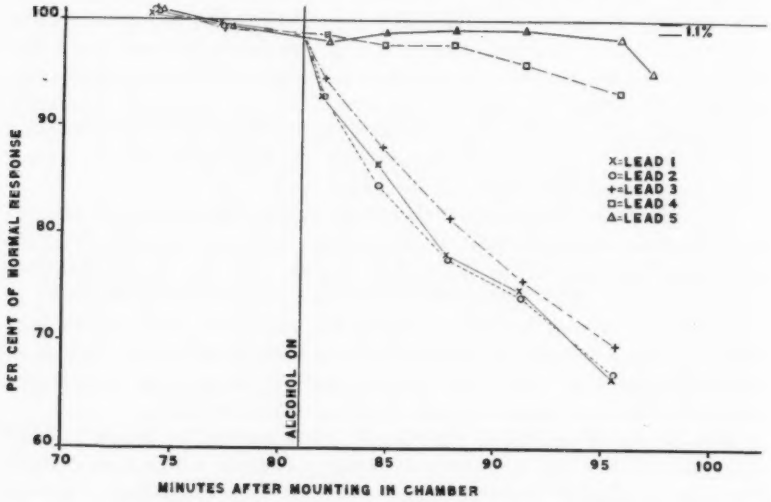

Fig. 2. September 11, 1924. Effect of alcohol vapor on electric response of excised peroneal nerve of cat. Leads *1*, *2* and *3* are in the narcotized region (cf. fig. 1). Note the parallel course of the depression of response at these points. Leads *4* and *5* are controls. Maximum percentage of locally blocked, and therefore uncontrolled fibers, is 1.1 per cent indicated in the figure by the distance between the short horizontal lines. See table 1 for data and experimental conditions. Note that base line is not the zero of ordinates.

experimental error, while the response at lead 2 is a little more depressed. This may be due to unequal narcotization, or to slight injury to the fascial sheath at 2, allowing more rapid penetration of the narcotic. The evidence against decrement is again clear. As before, the response at 5 begins to fall when that within the chamber has reached a level of about 70 per cent of normal. Conduction begins to fail in some of the fibers at this time, and as narcosis proceeds the response at all points falls rapidly until a steady state is reached at which the response at 5 is only about 8 per cent of its normal value. That all fibers do not eventually fail may be due to greater

resistance on the part of some fibers, or, more probably, to a weakening of the alcohol mixture by the prolonged bubbling. At this stage the striking feature is the comparatively strong response at lead 1, about 44 per cent of normal, as compared with 11 per cent at lead 2, and 5 per cent at lead 3. This is presumably the stage reached by Boruttau and Fröhlich (1904) in the experiments from which they infer decrement. No such inference is permissible, as our control shows that 92 per cent of the fibers have been blocked at some point in the chamber. If the particular point at which

TABLE 1

Effect of alcohol vapor on the electric response of excised nerve

	NARCOTIZING CHAMBER						CONTROL CHAMBERS			
Active lead	1		2		3		4		5	
Total resistance (ohms)	85,800 to 90,200		75,800 to 78,600		64,300 to 65,600		51,700 to 52,000		38,000	
	E.M.F.	Per cent normal	E.M.F.	Per cent normal	E.M.F.	Per cent normal	E.M.F.	Per cent normal	E.M.F.	Per cent normal
Control	602	100.5	592	100.6	582	101.0	560	100.6	359	100.8
Control	596	99.5	584	99.3	570	99.0	551	99.2	353	99.2
Average of controls	599	100.0	588	100.0	576	100.0	556	100.0	356	100.0
Minutes of narcosis:										
0.9 to 1.2	556	92.8	545	92.7	544	94.4	548	98.5	348	97.8
3.6 to 3.9	517	86.4	496	84.5	507	88.0	543	97.6	351	98.7
6.8 to 7.2	468	78.0	456	77.6	470	81.5	543	97.6	352	99.0
10.2 to 10.5	448	74.7	435	74.0	436	75.7	533	95.8	352	99.0
14.5 to 14.8	399	66.5	393	66.9	401	69.6	519	93.4	349	98.1
16.2									338	95.0

September 11, 1924. E.M.F. in arbitrary units. For galvanometer constants, method of calculation, and other tests on this same nerve prior to narcosis see Davis and Brunswick (1926). Distance between successive leads is 15 mm. Strength of stimulus = 14.3 Z units. Temperature = 19.5°—20.5°C. Narcotizing vapor obtained by bubbling 150 cc. of air per minute through about one centimeter depth of 6 per cent alcohol. Maximum percentage of fibers which may be locally blocked, as shown by shift-of-stimulus test, is 1.1 per cent.

block occurs is a chance phenomenon, depending on the presence of some slight trauma to reinforce the action of the narcotic or on some fortuitous unevenness in narcotization, then we should expect to find a fair proportion of fibers conducting as far as lead 1, a few to lead 2, and still fewer to lead 3. The graded responses observed may thus be explained quite simply on the basis of the number of fibers active at each point.

In figure 3 is also illustrated the partial recovery after substituting pure air for the narcotic. The return of the response at lead 5 exactly to its

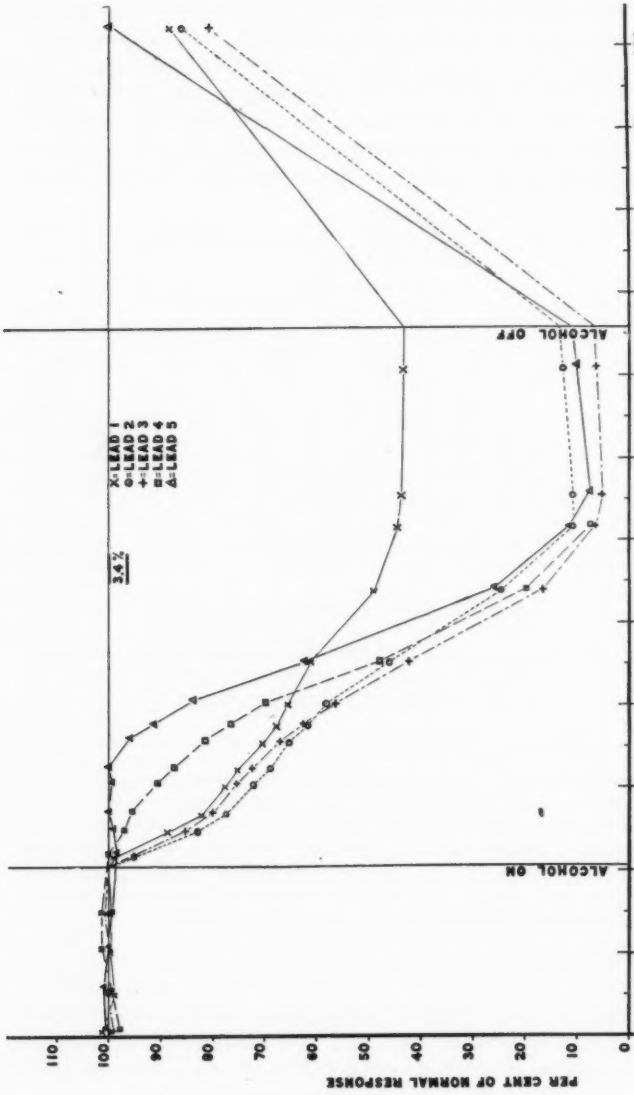

Fig. 3. February 3, 1925. Effect of alcohol vapor on electric response of excised peroneal nerve of cat. For method of calculation, galvanometer constants, and additional tests on this same nerve prior to narcosis see Davis and Brunswick (1926). Leads *1*, *2* and *3* are in the narcotized region; *4* and *5* are controls. Maximum percentage of locally blocked, and therefore uncontrolled fibers, is 3.4 per cent, indicated by the distance between the short horizontal lines on figure. Strength of stimulus = 12.6 Z units. Temperature = 20.4°—20.5°C. For arrangement of leads see figure 1.

original value after an hour and forty minutes is the significant point, showing that we are dealing with a reversible block, and that no progressive change was taking place in the condition of our control. In another experiment the recovery was followed still more completely, and the continuance of the original steady state and the parallelism of recovery at the various points in the narcotized region were verified.

Six other experiments, more or less completely controlled, confirm those here presented, and in no case were contradictory phenomena encountered. The depression was always identical, within limits of experimental error, at all three points in the narcotized region, as long as all fibers were conducting throughout their length.

It may be noted that in most of our experiments the control begins to fail when the response in the narcotized region reaches about 70 per cent of its normal value. This does not mean that conduction in a given fiber fails when its response is depressed to 70 per cent of normal. This figure is merely an average for all of the fibers. Those on the outside, which presumably fail first, being exposed to free access of the narcotic, are undoubtedly much more depressed, while those within may be only slightly affected. The nerve is of considerable diameter (1.3 to 1.7 mm.), and uniform distribution of the narcotic is not to be expected. A more gradual narcotization would probably show a greater depression before the control failed. Kato (1924) reaches a low level of 20 to 25 per cent in some experiments before any fibers fail entirely. This may be due to slower narcotization, or possibly to an ability of amphibian nerve to continue to function under more unfavorable conditions than mammalian nerve. This might be analogous to the greater resistance to the trauma of stretching described by Forbes and Ray (1923).

Discussion. The foregoing experiments, and the corresponding experiments of Kato, with which they are in entire agreement, indicate that as the impulse travels in uniformly narcotized nerve the electric response associated with the nerve impulse suffers no decrement (unless the decrement occurs in the first seven millimeters, where no tests were made). There is a depression of the electric response in the narcotized region dependent on the strength and the duration of action of the narcotic, but, within the above limits, independent of any length factor. If we accept the conclusion that in a narcotized region the velocity of conduction is constant though diminished, we may further conclude that the velocity of conduction in a narcotized region and the magnitude of the electric response vary in a parallel manner, as demanded by the membrane theory of conduction. While not a proof of the truth of the membrane theory, this is another point in its support, the theoretical prediction being verified experimentally. It is true that Achelis (1922) concludes that the velocity of the impulse suffers a decrement in a region of narcosis. His method

depends on measuring differences in the latent periods of electric responses of nerve or muscle as the stimulus is applied at one point or another in a narcotized region of the motor nerve. However, the probable error of his method is decidedly greater than Adrian's (1914b), and it is only by regarding his results statistically that a conclusion may be drawn from them. In addition to the great variability of his figures, even for normal nerve, he has not made full allowance for the consequences of current spread. His figures suggest that the stimuli from the last pair of electrodes excite the nerve outside the narcosis chamber, and those from the next to the last pair excite very near the lower end of the chamber. If this is true his test intended to rule out a differential effect of the narcotic on the first section of nerve near the cut end is invalid. As he clearly points out, such a differential effect would explain his results. It is not unreasonable to anticipate such differences, since we have shown (loc. cit.) that the electric response is reduced at least as far as 15 mm. from a crush (in mammalian nerve), and Kato has called attention to the great susceptibility of a damaged region of nerve to narcosis. It seems justifiable, therefore, to accept the conclusion of Adrian that the velocity of the impulse is reduced but constant in uniformly narcotized nerve.

It should be noted that the relationship between velocity and size of electric response does not depend on the assumption that the electric response is a measure of the intensity of the nerve impulse. It is a purely empirical relationship; and with it as additional support of the membrane theory, we may with increased confidence accept that theory as a working hypothesis, as suggested in the previous paper of this series, and employ the size of the electric response as an index of the intensity of the impulse. On this basis we now conclude that the *nerve impulse* undergoes no decrement in a region of narcosis, within the limits of length investigated. Furthermore, Adrian's conclusion (1914b) as to the independence of the intensity of the impulse and its velocity of transmission is no longer justified. Our results indicate a definite interdependence.

Our experiments, and the more varied experiments of Kato, furnish definite evidence as to the all-or-none character of the electric response. Not only is the response at a given point in the narcotized region independent of the length of narcotized nerve traversed by the impulse, but the response in the narcotized region may be depressed to 70 per cent of normal (according to Kato to 25 per cent) without affecting the magnitude of the control response beyond the narcotized region. The response here depends on local conditions and not on the magnitude of response at other points, at least if several millimeters of normal nerve intervene between narcotized region and control. This must mean that the energy of the electric response is derived from the fiber in the neighbor-

hood of the electrode, and the conception is untenable that the electric response is a transient electric current in an inert conductor of high resistance and distributed capacity (cf. Crehore and Williams, 1913).

If we accept the magnitude of the electric response as an index of the intensity of the nerve impulse we have a proof of the all-or-none character of the nerve impulse. This is important, since the only previous complete proof of the all-or-none relationship is that of Adrian (1912 and 1914a), in which he depends on the method of decrement in order to measure the intensity of the impulse. Lucas (1917) states that "it (decrement) is the basis of our only method of measurement. . . . . If we wish to measure the intensity of a nervous impulse we can set it to face a tract of nerve in which it will undergo a decrement, and determine how far it is able to travel before it is extinguished." From our experiments and Kato's, however, the conclusion is that the impulse does not suffer decrement, and Kato has shown that previous experimental evidence underlying the conception of decrement is unsound. The argument of Lucas and Adrian falls to pieces unless it can be proved by some new method that decrement occurs over the short lengths of nerve employed by Adrian; and failing this we must substitute the evidence and argument outlined above as the basis of the all-or-none law.

Throughout the above discussion we have limited our conclusions to the portion of a nerve between seven and thirty-seven millimeters from the beginning of a narcotized region, representing the actual positions of our electrodes. There is no reason to believe that these conclusions may not be extended to an indefinitely long stretch of nerve. In the case of shorter lengths than seven millimeters, however, we must be more guarded. Kato does not hesitate to extend his conception of decrementless conduction to all lengths of nerve, provided only narcosis be uniform. The anomalous results, namely longer continued conduction, with small narcotizing chambers (6 mm. or less in diameter) he attributes to diffusion of the narcotic along the nerve out of the chamber, and a consequent failure to attain full depth of narcosis within 3 mm. of the wall of the chamber. It seems to us that while qualitatively diffusion undoubtedly occurs, it is dangerous to attribute so great a quantitative effect to it without direct evidence in support. It would seem on general principles that, given a large and freely circulating supply of narcotic such as alcohol vapor, the diffusion gradient of alcohol would lie almost entirely *outside* the chamber, and such a "draining" as Kato pictures would hardly be significant for a distance within the chamber greater than the diameter of the nerve. It is futile to argue the point closely, as the magnitude of the effect obviously depends on the relative rate of diffusion of narcotic longitudinally and transversely in the nerve trunk. If the longitudinal rate is very markedly greater than the transverse, as may possibly be the case, Kato's supposition may be correct.

We would emphasize, however, that Kato's explanation in terms of diffusion is still hypothesis, and we suggest the alternative possibility that on passing from normal to narcotized nerve *the impulse does not fall immediately to the lower level characteristic of narcosis, but is conducted with a decrement for a short distance before reaching its new level.* Conversely, we would suppose that on emerging from the narcotized region it would not resume its full strength instantaneously, but would rise rapidly at first and then more gradually, being conducted with an "increment" until the new level was reached. This, be it understood, we believe would occur even assuming no diffusion of narcotic and a perfectly sharp transition between normal and narcotized nerve. Figure 4 illustrates the contrast.

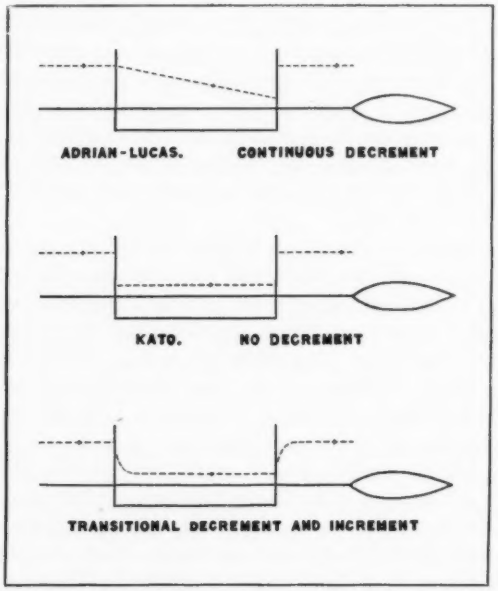

Fig. 4. The shape of the curve representing transitional decrement is purely tentative. It is given a logarithmic character as a first approximation because the fall of electrical potential along a poorly insulated conductor follows a logarithmic law, and because we believe the mechanism of transmission involves such a system. The increment is tentatively represented as the converse of the decrement. Modification will be necessary in the future, particularly at the junction points between normal and narcotized regions.

The distance from the wall at which the minimum level is reached is indefinite, but is apparently less than 7 mm., perhaps much less.

Lucas' representation of decrement as linear was avowedly tentative also; but his conception differs fundamentally from ours in the continuous character of the fall, failing to reach a definite minimum value.

Our experiments indicate that the electric response, and presumably therefore the impulse, has reached its new level at a point seven millimeters from the wall. Whether decrement extends as far as six millimeters must still be decided, and care must be taken to distinguish decrement in the true sense from the mere gradient of narcosis which Kato assumes.

In opposition to Kato's view we may cite the observation of Fröhlich (1904, pp. 170–171), who tested the excitability of a nerve immediately beyond the wall of his narcotizing chamber. The wall was 2 mm. thick and ether vapor was allowed to act for an hour. The chamber itself was only 1 mm. in length, and the vapor was strong enough to suspend conduction in fifteen minutes; yet no rise in the threshold outside was observed throughout the entire experiment. This indicates that diffusion is far less significant than Kato assumes.[2]

Definite evidence of decrement in the first few millimeters of a region of impaired conduction is presented by Drury (1925). He applied pressure to a strip of dog's auricular muscle (*in situ*) and measured the velocity of conduction of the excitation waves and also observed the size and character of the electric response at various points. The electrodes between which velocity was measured were one, five and nine millimeters from the beginning of the region of compression. His figures show beyond question a more rapid conduction between the first and second points than between second and third. Likewise the electric responses become progressively smaller and more atypical in character. Uniformity of pressure is satisfactorily controlled. Cardiac tissue behaves functionally as a single unit; and in this experiment there is no question of diffusion of narcotic, nor of spread of stimulus. The fundamental identity of the conduction process in muscle and nerve may be regarded as proven, so that from Drury's work we may safely infer decremental conduction, at least for a limited distance, in nerve.

The following general considerations of the nerve impulse and its transmission may serve to reconcile the apparently conflicting lines of evidence concerning decrement by indicating how initial decrement followed by a constant level of intensity might be predicted *a priori*.

Certain important attributes of the impulse we may regard as definitely established; namely, *a*, the association with it of an electric disturbance by which the time of arrival, whether or not the intensity, of the impulse may be measured; *b*, the discontinuous character of nerve response, evidenced by the discontinuous electric response and by the refractory phase; *c*, the general order of the velocity of conduction; and *d*, the possibility of exciting any point on the nerve by a variety of agents and obtain-

[2] The phenomenon of Wedensky inhibition at a region of partial narcosis is very difficult to interpret unless we assume decremental conduction for at least a limited distance. Experimental work bearing on this point is now in progress.

ing an identical response. To this list we may now add *e*, the all-or-none character of the electric response. These characteristics point strongly to the conclusion that the nerve impulse depends on a reaction which, in kind and probably in intensity, is characteristic of the nerve fiber and not of the stimulus. The nerve impulse itself we may define as a *conducted tendency to excite*, since we recognize it ultimately only by the fact of its being conducted and inducing a state of excitation in some tissue. The intensity of the impulse should logically be measured in terms of that characteristic or result of the reaction, which tends to induce the reaction at a neighboring point. For example, in Lucas's gunpowder-train analogy the intensity index will be the temperature developed by the combustion at a given point. Nerve conduction might conceivably depend on a chemical reaction initiated at each point by certain products of the reaction which diffuse down the nerve from the active region. In that case the intensity will be represented by the concentration of the exciting substances.

In general for a reaction at one point to initiate a reaction at another point, there must be a transfer of either energy or material, and such a transfer will occur only if there is a gradient of energy.[3] The gradient might be electrical, measurable as a difference of potential between active and inactive regions and giving rise to current flow, or it might be a concentration gradient giving rise to diffusion.

We may therefore examine the general case of an energy gradient progressively reinforced by a local reaction and consequently enabled to propagate itself indefinitely through the system in which it exists. The level of energy at a given point in advance of the reaction will depend on the distance to the point in question, the energy level attained at the active region, and the laws of spread of that type of energy in the particular system. A time factor will usually appear in these laws, but in all cases there will be an attenuation of the energy with distance. Furthermore, at an active point, the level of energy will depend on two factors: the rate of energy development at that point, and the rate of transmission to and from other points.

Imagine the case of a nerve treated locally with some non-diffusing narcotic which prevents the characteristic reaction from occurring, but which does not appreciably alter the conditions of spread of the particular form of energy which is responsible for the transmission. When the last

[3] The term gradient of energy is here used in the sense of a thermal gradient or diffusion gradient to express the relation of a high potential of energy at one point, a low potential at another, and intermediate potentials at intermediate points. The implication is that the system is capable of doing work by virtue of the differences in temperature, concentration, electric potential, or whatever form of energy is concerned.

section of normal nerve becomes active a gradient of energy will exist into the narcotized region, and may have a value at the far end sufficient to initiate the characteristic reaction of the normal nerve at that point. This is essentially the hypothesis first proposed by Werigo (1899). The nerve will have conducted across the narcotized area even though the characteristic reaction will not have occurred in that region. And since we define the nerve impulse as a "tendency to excite," it is proper to measure its intensity at various points in the narcotized "inactive" region. Its intensity at each point will depend on the character of the energy gradient. Inevitably in a material system there will be energy loss[4] as the energy spreads, and it will spread only by virtue of being at a higher potential at each point than at the next successive point. This attenuation of intensity with distance is what we mean by "conduction with a decrement."

The degree of decrement, that is, the abruptness with which the intensity of the impulse falls off, will depend on the rate of energy loss along the fiber. It may be that this is increased by the narcotic, and that the higher the concentration of narcotic the greater the decrement. Probably when the structure of the nerve is destroyed, as by ligation, decrement becomes very great indeed.

The intensity of the impulse will be the same at all points in a normally conducting nerve, since an equilibrium will be reached between the energy losses in transmission along a unit length of nerve and the energy developed by the characteristic reaction of that unit. If for any reason (e.g., narcosis, cold, electrotonus, etc.) the energy development per unit length is diminished, equilibrium may eventually be established at a lower level; but, like every dynamic equilibrium, it will not be established at once, and the effect of the normal response will be perceptible for a definite distance into a partially narcotized region until the new equilibrium is reached. This will give an initial decrement on entering the region of narcosis, a steady equilibrium level characteristic of the degree of narcosis, and finally an increment on emerging from the narcotized region. All of this is illustrated in figure 4.

While we may thus with certainty predict conduction with a decrement under certain conditions, whatever be the mechanism of conduction, we cannot specify the length of nerve over which it will be manifested. Experiments of Kato (1924) and of Rajmist (reported by Werigo, 1899) show a correlation between the length of nerve and the degree of narcosis required to suspend conduction only when the length of nerve is less than 5 or 6 mm. This suggests that decremental conduction may occur for such a distance. On the other hand, if Kato's hypothesis of diffusion of

[4] By the term "energy loss" we do not imply destruction of energy or even its escape from the system, but rather a degradation of energy so that the system becomes less capable of doing work.

narcotic is the correct explanation of the above results, the extent of decrement may be very much less than the 5 or 6 mm.; but it seems almost certain that decrement does extend over distances as short as those involved in synaptic junctions. Indeed we might tentatively regard a synapse as a section of nerve which does not undergo the characteristic reaction, but simply provides a passive structure along which the impulse spreads with a decrement. There is no need to abandon the theoretical explanations of central nervous system function based on synaptic decrement merely because the idea of continuous decrement over unlimited lengths of nerve has been proved untrue.

The discussion of decrement so far is independent of any special theory of the mechanism of conduction; and it may be well to illustrate in terms of the membrane theory. The characteristic reaction is assumed to be an increase in permeability of some polarized semipermeable membrane. This is initiated by anything which sufficiently depolarizes the membrane, and its result is to establish a difference of electric potential between active and inactive regions. This gives the energy gradient, and as a result electricity flows. This depolarizes the next region, and the cycle is repeated. The intensity of the nerve impulse is the depolarizing effectiveness of the action current. An analysis in these terms has already been made in a previous paper (Davis and Brunswick, 1926).

If we accept this theory it is possible to determine approximately from the data of Bishop, Erlanger and Gasser (1925) the maximum length of nerve over which conduction with a decrement could occur. These workers record without instrumental lag or overshoot the changes of potential at a given point on the nerve. The first change is presumably due to "spread" from the approaching wave of activity, and then follows a combination of "spread" plus the activity developing under the electrode. The potential under the electrode rises to 1.5 mv. before activity begins to develop there, according to their interpretation, requiring 0.075σ[5] to rise from not over one-tenth that value. We may tentatively assume that this represents the potential required for excitation. Assuming the velocity of conduction to be 40 meters per second (Erlanger and Gasser, 1924) we calculate that the potential gradient in advance of a region of activity is of such steepness that the potential falls to one-tenth at a distance of 3.0 mm. The maximum potential developed at a given point is 30 mv. The distance in advance of such a point of maximum activity at which the potential would be 1.5 mv., assuming that the intermediate

[5] It should be noted that this figure arrived at by Bishop, Erlanger and Gasser in their preliminary statement is small in comparison with the probable error of measurement and the various corrections which must be applied to the data. It is to be regarded as indicating merely the order of magnitude of the maximum length of the "foot" of the action current.

points do not respond at all, is about 4 mm., calculating from the above data by the logarithmic law governing the spread of electric potential (cf. fig. 4). Consequently the impulse might "jump over" an irresponsive region 4 mm. in length. If the region to be "jumped" responds somewhat, but not strongly enough for continued propagation, the possible length of decremental conduction might well be increased to 5 or 6 mm. The distance is of the same order of magnitude as that indicated by Rajmist's and Kato's experiments described above.

Since according to this theory the energy gradient responsible for transmission is electrical, certain interesting possibilities appear concerning the spread of electrical stimuli. The stimulating cathode lowers the electrical potential locally on the nerve fibers. A gradient is, therefore, established away from the electrode in both directions. This gradient is in the same direction as the action current resulting from the stimulation, and the action current will reënforce it. There will be no way of distinguishing between these two gradients at a given point, and the intensity of the impulse, being measured in terms of the intensity of the electrical flow, will be supernormal within the range of influence of the cathode.[6] That is, the impulse here has a *greater* intensity than after the equilibrium condition characteristic of transmission along normal nerve has been reached; and until equilibrium is reached the impulse is conducted with a decrement. The velocity of conduction near the stimulus would be greater than normal; and the net results would be difficult to distinguish in practice from "spread of stimulus" in Kato's sense,—and for most purposes it is not necessary to make any distinction.

The iron wire model of nerve transmission elaborated by Lillie (1923) is clearly a case of the so-called "membrane" mechanism. The chief differences between the behavior of the model and of nerve have been in the matter of conduction with a decrement. In the model it is possible to show decremental conduction during the relative refractory period. The second impulse, started with more or less difficulty, travels more and more slowly as it progresses, and finally dies out. In the nerve, however, a second impulse as it lags farther behind its predecessor increases its size and accelerates by degrees (Gasser and Erlanger, 1925) until size and velocity become normal. On the other hand, nothing corresponding to the decrement in narcosis has been shown by the iron wire model. Varying the strength of the nitric acid alters the rate of conduction, but causes a

[6] The gradient from the stimulus is the one which must be established along the limiting semipermeable membrane of each fiber, and which, according to the Nernst-Hill theory of excitation, is responsible for the movement of ions that depolarizes the membrane and causes excitation. It is more or less independent of certain other types of "current escape" or "artefact" which travel over the nerve as a whole and may distort records of the electric response of the nerve.

uniform depression of rate—not a decrement. We have now shown that nerve behaves in like manner when narcotized; and we believe the phenomena in the refractory phase of the model to be capable of simple explanation along the lines of stimulus spread suggested above.

In the first place, too close a correspondence must not be expected, since the recovery in the model is vastly slower than in nerve, while the velocity of conduction is roughly the same. The second nerve impulse, traveling more slowly than the first, finds the nerve more and more nearly recovered at each point, capable of giving a larger response which in turn causes more rapid transmission. With the model it requires a considerable difference in velocity between the first and second impulses for the second to find the wire appreciably more recovered at each successive point. The comparison between the two systems is not an accurate one unless the wire is allowed to recover so far that the second impulse will travel for an indefinite distance. Then parallel behavior might be anticipated although the acceleration in the wire would be slight. The initiation of an early second impulse which travels with a decrement and is soon extinguished corresponds to stimulating the nerve toward the end of its absolute refractory period when, if it conducts at all, it will not conduct a second response far enough to be detected.[7] The limited conduction with a decrement observed in the wire model presumably represents a reënforcement by the stimulating current of an impulse too weak to propagate itself in the face of the existing threshold of excitation. The conditions for such reënforcement are ideal. Stimulation is usually accomplished by touching the passive iron with a piece of zinc, forming a battery with the iron and acid. A gradient of potential is established along the iron wire between wire and acid. Because of the very low resistance of the wire and the fairly low resistance of the acid this gradient extends for a considerable distance along the wire. In other words, the current flow in the zinc-acid-iron-zinc circuit is intense enough at considerable distance from the point of contact of zinc and iron to cause appreciable stimulating effect. Near the contact the current is most intense and causes stimulation first. The potential difference between active and passive iron now comes into play and reënforces the current flow in the previously existing circuits. A second battery has been introduced in parallel with the first. The active state spreads in the usual way, and, as the advanced edge of the region of activity recedes from the zinc, the effect of the latter is progressively reduced. Eventually the sum of the two potentials is no longer sufficient to cause excitation. Of course this condition is not reached abruptly, but the effect of the zinc wanes gradually as the active region spreads away from it; and the impulse is conducted with a decrement.

[7] This correspondence has been suggested by Lillie; 1925, Journ. Gen. Physiol., vii, 494.

That the potential due to the zinc summates with the "action current" of the model is indicated by the observation of Dériaud and Monnier (1925) who, while recording such "action current," note that "le contact de la lame de zinc qui amorce la propagation du phénoméne produit une variation électrique." This appears in their figure as a displacement of the base line equal in extent to one-sixth of the maximum height of the excursion of which it forms the beginning.

In nerve the steepness of the gradient is apparently considerable, so that the distances over which decrement is appreciable are comparatively small, as pointed out above. An electrical gradient of this type is represented by a logarithmic curve, not a straight line, and therefore we have tentatively chosen a curve of this type for our schematic diagram (fig. 4). The steepness of such a gradient, or better its attenuation, depends directly on the resistances in the circuit (inside and outside the nerve) and on the "leakance" or readiness with which a difference of potential discharges across the "membrane." If the "membrane" is destroyed, as would be the case in crushing, the leakance becomes very great and the attenuation also becomes very great. Speaking loosely, we may say that the two sides of the membrane are short circuited. The net result is that the gradient does not extend beyond the crush. This explains why a crush, ligature, or other damage to the nerve, hinders spread of stimulus and cannot be used as a control for this phenomenon, as Kato found empirically. It may well be, furthermore, that deep narcosis acts in somewhat the same way and thus hinders spread of stimulus out of a narcotized region.

SUMMARY

The electric responses from various points on a nerve were recorded by a method previously described, and the effect of alcohol vapor on the responses investigated. Careful control was employed to insure that approximately all fibers conducted the whole length of the nerve throughout the experiment, and therefore the observed response of the nerve at a given point represented the average response of the individual fibers. At the three points tested within the narcotized region the relative depressions of the electric responses were identical for a given degree of narcosis. (No point tested was less than 7 mm. from the chamber wall.)

This result is in entire agreement with the recent work of Kato, although the experiments were performed without knowledge of that work; and we conclude that the electric response does not suffer decrement as the impulse is conducted through such a region of narcosis. This is contrary to the classical theory of conduction with a decrement. The chief evidence supporting that theory and Kato's further experiments and criticism of the old evidence are reviewed briefly. "Spread of stimulus" as a source of error is particularly emphasized. By this is meant the

occurrence of stimulation at some distance from the electrodes. It is not permissible to crush the nerve near the electrode and, finding no excitation beyond the crush, to conclude that spread did not occur to this extent prior to crushing.

Our results and Kato's revealing a uniform depression of electric response in a narcotized region are of interest in relation to Adrian's earlier work showing a constant although reduced velocity of conduction in such a region. We now conclude that there is a parallelism between the size of electric response and the velocity of conduction, even in narcotized nerve. This is the relationship predicted by the "membrane" theory of nerve conduction, and we interpret our results as giving additional experimental support to this theory. It is further shown that, accepting this theory, the electric response may be used as a measure of the intensity of the nerve impulse.

We consider that our work and that of Kato makes the old theory of "decremental conduction" untenable. Therefore the proof by Adrian of the all-or-none law is not valid, since he assumed decremental conduction in his method of measuring the intensity of the impulse. Our experiment,

Fig. 5

however, showing depression of electric response within the chamber but normal response beyond, is a direct proof of the all-or-none character of the electric response. If the electric response may be taken as an index of the intensity of the nerve impulse, we conclude that the impulse itself obeys the all-or-none law.

The mechanism of conduction is considered from a general theoretical point of view, and it is concluded that transmission of the impulse from point to point must depend on a gradient of energy from the active to the as yet inactive region. The intensity of the impulse should logically be measured in terms of this energy. In passing from tissue responding normally to tissue depressed in its response, the intensity of the impulse cannot change abruptly from one level to another. There must be a *transitional decrement*, extending as far into the depressed region as the gradient from the higher level of energy developed by the normal region is perceptible. Conversely, there must be a transitional increment where the impulse emerges from a region of depressed response. We represent the behavior of the impulse by the accompanying diagram (fig. 5). The distance over which decrement extends is undetermined as yet, except that it must be less than 7 mm. Since decrement probably extends over dis-

tances as short as synaptic junctions, there is no logical need to abandon the theoretical explanations of central nervous system function based on synaptic decrement.

Conduction with a decrement in the refractory phase, shown by Lillie's artificial nerve model, is interpreted as depending on a reënforcement of the local action current by the stimulating current. The prevention of spread of stimulus in a nerve by a crush is tentatively explained as due to interference by the crush with a similar reënforcement phenomenon.

We wish to thank Miss Carola Eliot for assistance in preparing the manuscript for publication.

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THE PRODUCTION OF SOME OF THE PHENOMENA PECULIAR TO PARATHYROID OVERDOSAGE IN DOGS BY MEANS OF CERTAIN INORGANIC SALTS

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Received for publication January 11, 1926

It has been shown elsewhere (1), (2) that dogs are peculiarly sensitive to injections of the parathyroid hormone. Repeated injections of small doses at intervals of a few hours invariably lead to the manifestation of a typical train of symptoms which result in death in about 48 hours. A study of the blood chemistry as influenced by parathyroid hormone overdosage has revealed that blood serum calcium, whole blood inorganic phosphorus (as determined by the Briggs method) (3) urea and non-protein nitrogen are altered in a manner which is characteristic for each of these constituents (2). In the first 24 hours of such experiments there is only one outstanding change noted in the blood chemistry. This change consists in the gradual establishment of a condition of marked hypercalcemia (20 mgm. per 100 cc. serum). There may be a slight increase in whole blood inorganic phosphorus, urea and non-protein nitrogen in the latter part of this period. In the first few hours of the same period there is, as a rule, a slight decrease in the hemoglobin value which is indicative of an increased plasma volume. The second 24-hour period or the second half of such experiments is the period in which urgent symptoms become manifested and coincident with the development of these symptoms, the blood chemistry shows characteristic changes. These consist of a fall in blood serum calcium, a marked rise in inorganic phosphorus and a gradual and uniform increase in urea and non-protein nitrogen. The inorganic phosphorus rises gradually at first but in the last two to four hours this constituent increases very abruptly. The increase in urea and non-protein nitrogen is of a nature which indicates that kidney function is ceasing. (Compare urea and non-protein nitrogen curves for parathyroid overdosage and similar curves for retention due to ureter ligation. Charts 1 and 2.) Associated with these changes there is in the last stage an acidosis. Death then is due to one or more if not all of these changes, namely, hypercalcemia, hyperphosphatemia, cessation of kidney function and acidosis. It was thought that this set of conditions could be experimentally produced by the administration of certain mineral salts. With this object in view the present investigation was undertaken.

At death from parathyroid hormone overdosage, the characteristic finding is intense hyperemia and venous engorgement of the stomach and intestines. Considerable blood or altered blood is found in the stomach and intestines and petechial hemorrhages are seen on the mucosa. On section the capillaries are seen to be much distended. The bleeding is probably of the nature of a diapedesis of red cells through the endothelial walls of the capillaries. See figure 2.

METHODS. Normal dogs were used. Salt solutions were administered by injection into a leg vein. Inorganic phosphorus was determined in whole blood by the Briggs method and calcium was estimated in blood serum by the Clark-Collip modification of the Tisdall method.

RESULTS. The experimental results are shown in the form of charts and protocols.

DISCUSSION OF RESULTS: *Hypercalcemia due to intravenous injection of calcium chloride.* It is very difficult to produce and maintain a condition of uniform hypercalcemia by the intravenous injection of calcium chloride. Salveson (5) and others have shown that injected calcium leaves the circulation very rapidly. This we have confirmed. By the repeated injection, by the intravenous route, of calcium chloride (the injections being made very slowly) the blood serum calcium can be maintained at approximately 20 mgm. per 100 cc. for many hours. Under these conditions there is noted a slight rise in inorganic phosphorus and an increase in the viscosity of the blood. The condition is well borne, however, and no urgent symptoms have appeared in our experiments.

Hypercalcemia due to oral administration of calcium lactate. Hjort (6) claims that calcium lactate administered orally causes a very definite increase in blood serum calcium. In our experiments calcium lactate has been administered by stomach tube in 10 gram doses at frequent intervals and calcium chloride in 5 gram doses. In one instance which we take to be an exceptional experiment, the treatment with calcium lactate resulted in the manifestation of profound hypercalcemia and death in 5 hours (chart 3). The results of other experiments of this type are shown in chart 4.

The inorganic phosphorus of the whole blood in these experiments, with the one exception, increased very little. An increase in the viscosity of the blood was noted in most instances.

Hypercalcemia and hyperphosphatemia due to intravenous injection of calcium chloride and acid sodium phosphate. Hypercalcemia due to the intravenous injection of calcium chloride alone is well borne so also is hyperphosphatemia due to the intravenous injection of acid sodium phosphate. Hyperphosphatemia produced by alkaline phosphate lowers blood serum calcium and produces fatal tetany, as Binger (7) and others have shown. It has been found that this result may be offset by the repeated injection

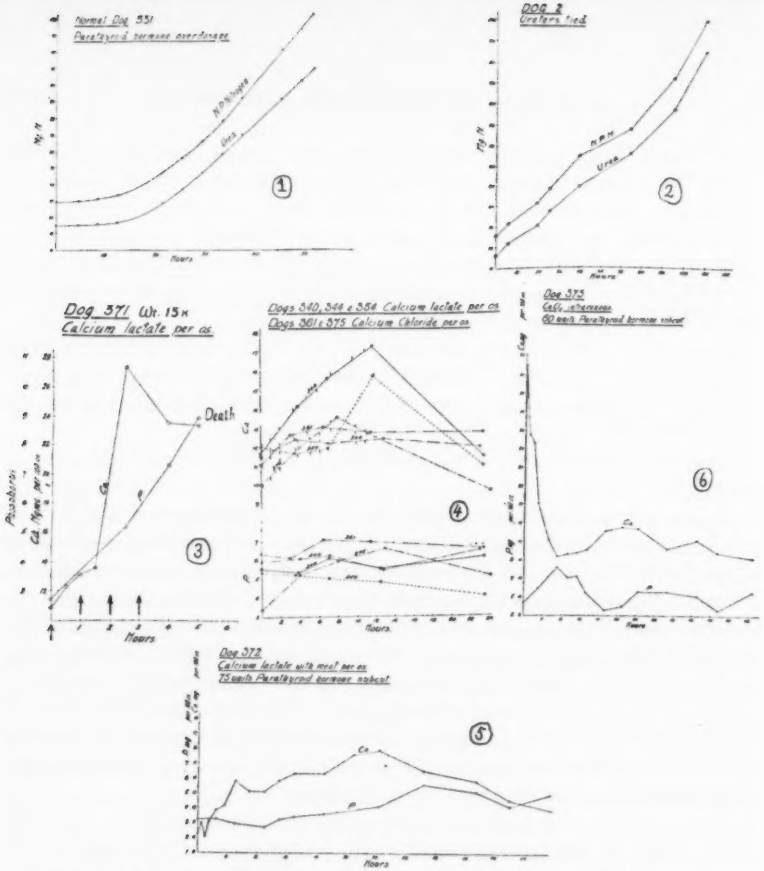

Chart 1. Dog 331, ♂, 18.5 kilos. Showing effect of parathyroid hormone overdosage upon the urea and non-protein nitrogen curves. The curves compare favorably with those for retention. See chart 2.

Chart 2. Dog 2, ♀, 16.5 kilos. Both ureters ligated under ether anesthesia. Showing effect of suppression of kidney function upon blood urea and non-protein nitrogen.

Chart 3. Dog 371, ♀, 15 kilos. Ten grams of calcium lactate given by stomach tube at times indicated by arrows on chart. Profound hypercalcemia followed and death ensued. The blood became very viscous and it was difficult to separate the serum therefrom.

Chart 4. Dog 340, ♂, 18.5 kilos; dog 344, ♂, 12.5 kilos; dog 354, ♂, 18 kilos—thyroparathyroidectomized; dog 361, ♂, 10 kilos; dog 375, ♂, 11 kilos.

Dogs 340, 344, 354, received 10 grams calcium lactate in 500 cc. of water at times indicated on chart. Dogs 361 and 375 received 5 grams of calcium chloride in 400 cc. of water at times indicated on chart.

Chart 5. Dog 372, ♂, 16.5 kilos. Seventy-five units parathyroid hormone injected subcutaneously. At every hour for 5 hours 10 grams of calciumlactate fed with lean meat. Curves show effect upon blood serum calcium and whole blood inorganic phosphorus.

Chart 6. Dog 373, ♀, 14.5 kilos. Forty cubic centimeters of 5 per cent $CaCl_2$ injected intravenously followed immediately by 60 units of parathyroid hormone subcutaneously. Curves show subsequent effect upon blood serum calcium and whole blood inorganic phosphorus. Note the reinforcing action of the calcium lactate per os upon the calcium curve.

of calcium chloride in adequate amount. Under these circumstances both the injected phosphate and calcium tend to leave the circulation very rapidly, being eliminated in all probability as Ca_3PO_4. In all of the conditions above mentioned kidney function appears not to be impaired.

When calcium chloride and acid sodium phosphate are administered by intravenous injection into the same animals the result is very different. The changes associated with the preterminal hours of parathyroid hormone overdosage are produced almost immediately and death results very shortly. The blood quickly becomes thick and viscous, the circulation fails and collapse soon follows. If the amount of calcium chloride and sodium phosphate solution used is not excessive the experiment may be prolonged and an even closer parallelism with parathyroid overdosage

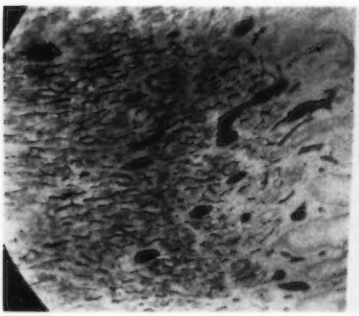

Fig. 1

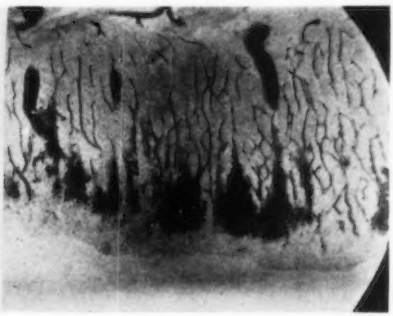

Fig. 2

Fig. 1. Photomicrograph of mucosa of stomach of dog dying from parathyroid hormone overdosage. Note the dilated capillaries and venules, also the hemorrhagic areas. Section prepared by courtesy of Dr. R. F. Shaner.

Fig. 2. Photomicrograph of mucosa of stomach of dog dying from $CaCl_2$ and NaH_2PO_4 injections. Contrast with figure 1. Section prepared by courtesy of Dr. R. F. Shaner.

phenomena obtained. Kidney function is decreased and at death there is noted a definite retention of urea and non-protein nitrogen in the blood. The post-mortem findings, if the experiment has been prolonged for many hours, are almost identical with those present at death in parathyroid hormone overdosage experiments. The characteristic features here as outlined in a previous paragraph, capillary dilatation and hemorrhage into the mucosa of the stomach and intestine, are present in just as typical a manner. See photomicrograph figures 1 and 2.

SUMMARY

Some characteristic phenomena of parathyroid overdosage as manifested in dogs have been produced by the intravenous injection of adequate amounts of calcium chloride and acid sodium phosphate.

The intravenous injection of solutions of calcium chloride or of either acid or alkaline phosphate alone have not produced this result.

Repeated administrations of calcium lactate or chloride to fasting dogs may cause a temporary rise in blood calcium. Profound hypercalcemia and death resulted in 1 experiment in 6.

It is a pleasure to acknowledge the assistance of Drs. E. P. Clark and N. J. Minnish who carried out the blood analyses in the experiments herein reported.

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PROTOCOLS

DOG NUMBER	SEX	WEIGHT	TIME	REMARKS	UREA N	N.P.N.	Ca	P
		kgm.						
367	♂	18.5	9:30 a.m.	180 mgm. morphia				
			11:00 a.m.		13.4	30.8		3.7
			11:15–11:20 a.m.	25 cc. 5 per cent $CaCl_2$ intravenous				
			11:25 a.m.	7 cc. 20 per cent NaH_2PO_4 intravenous				
			12:30 p.m.	3 cc. 20 per cent NaH_2PO_4 intravenous				
			2:00 p.m.	5 cc. 20 per cent NaH_2PO_4 intravenous				
			2:15 p.m.	27 cc. 5 per cent $CaCl_2$ intravenous				
			3:15 p.m.		18.3	39.2	16.1	10.5
			4:30 p.m.	10 cc. 20 per cent NaH_2PO_4 intravenous				
			6:00 p.m.		23.8	44.8		12.0
			11:00 p.m.	Death	39.2	75.0	15.2	14.0
			Post-mortem: Stomach and intestines markedly engorged. Mucosa diffusely hemorrhagic. Petechial hemorrhages present. Blood stained fluid and coffee ground material in stomach and lumen of intestine. Spleen hemorrhagic and engorged. Kidney normal. About 200 cc. urine in bladder. No urine passed during experiment.					
366	♂	22	12:15 p.m.		12	28.8		
			12:15–35 p.m.	44 cc. 5 per cent $CaCl_2$ injected				
			12:45 p.m.	35 cc. 20 per cent NaH_2PO_4 injected				
			12:50 p.m.				17.1	36.4
			3:05–3:20 p.m.	22 cc. $CaCl_2$ injected				
			3:25 p.m.	15 cc. 20 per cent NaH_2PO_4 injected				
			4:15 p.m.	Collapse	19.9	42.8		18.5
			Post-mortem: Slight congestion of bowel most marked in colon. Spleen hemorrhagic. Blood so viscous serum could not be separated for calcium determination.					

PROTOCOLS—*Continued*

DOG NUMBER	SEX	WEIGHT	TIME	REMARKS	UREA N	N.P.N.	Ca	P
		kgm.						
362	♂	45	Amytol anesthesia					
			2:00 p.m.				11.2	3.6
			2:05–2:40 p.m.	40 cc. 10 per cent $CaCl_2$ injected				
			2:40 p.m.				22.1	
			2:45–3:00 p.m.	75 cc. 20 per cent NaH_2PO_4 injected				
			3:00–3:15 p.m.	20 cc. 5 per cent $CaCl_2$ injected				
			3:25 p.m.	35 cc. 20 per cent NaH_2PO_4 injected				
			3:30 p.m.	10 cc. 5 per cent $CaCl_2$ injected				
			3:55 p.m.	10 cc. 5 per cent $CaCl_2$ injected				
			4:05 p.m.	Sudden collapse. Hemoglobin 8 per cent increase			31.8	50
			Post-mortem: Bladder full. Alimentary canal congested. Spleen congested and hemorrhagic, meninges congested.					
360	♂	17	12:15 p.m.	15 cc. 10 per cent $CaCl_2$ injected				
			12:20 p.m.	25 cc. 20 per cent NaH_2PO_4 injected				
			2:30 p.m.	50 cc. 2.5 per cent $CaCl_2$ injected				
			2:40 p.m.	20 cc. 20 per cent NaH_2PO_4 injected				
			2:40 p.m.	Collapse and death				
358	♂	24	4:30 p.m.		12.5	30.0		3.6
			4:30 p.m.	23 cc. 20 per cent NaH_2PO_4 injected.				
			4:35 p.m.					40.8
			12:00 p.m.		22.0			3.8
			10:30 a.m.		18.0	42.0		3.0
			10:30 a.m.	25 cc. 20 per cent NaH_2PO_4 injected				
			12:40 p.m.				10.1	7.0
			12:40 p.m.	12 cc. 20 per cent NaH_2PO_4 injected				
			3:30 p.m.	15 cc. 20 per cent NaH_2PO_4 injected				
			5:30 p.m.			34		4.2
			10:00 p.m.	Normal			11.4	3.0

PROTOCOLS—*Continued*

DOG NUMBER	SEX	WEIGHT	TIME	REMARKS	UREA N	N.P.N.	Ca	P
		kgm.						
357	♂	22	10:35 a.m.	20 cc. 10 per cent $CaCl_2$ injected				
			10:45 a.m.	25 cc. 20 per cent NaH_2PO_4 injected				
			10:50 a.m.				24.5	20.8
			12:00 a.m.	15 cc. 20 per cent NaH_2PO_4 injected				
			12:15 p.m.	Vomiting and diarrhea				
			1:00 p.m.	10 cc. 20 per cent NaH_2PO_4 injected				
			1:30 p.m.	Bloody stool				
			2:00 p.m.				12.7	17.0
			2:00 p.m.	15 cc. 10 per cent $CaCl_2$ injected				
			2:30 p.m.	10 cc. 20 per cent NaH_2PO_4 injected.				
			3:40 p.m.	Collapse. Blood very viscous			14.8	20.8
			Post-mortem: Diffuse hemorrhagic condition of mucosa of stomach and intestine.					
365	♂	28	11:50 a.m.		16.8	30.2	11.0	2.1
			12:00 a.m.	30 cc. 5 per cent $CaCl_2$ injected				
			12:10 p.m.	10 cc. 5 per cent $CaCl_2$ injected				
			12:15–12:40 p.m.	60 cc. 6 per cent sodium phosphate mixture pH 7.3				
			12:40 p.m.				13.4	14.3
			2:15 p.m.				11.9	6.9
			2:20–2:30 p.m.	20 cc. 5 per cent $CaCl_2$ injected				
			2:30–2:45 p.m.	50 cc. 6 per cent phosphate injected pH 7.3				
			4:20 p.m.				10.6	10.2
			4:30 p.m.	20 cc. 5 per cent $CaCl_2$ injected Vomited during injection				
			4:45 p.m.	55 cc. 6 per cent phosphate injected pH 7.3				
			5:00 p.m.	15 cc. 5 per cent $CaCl_2$ injected				
			6:00 p.m.	Hyperpnea				
			6:50 p.m.	Fatal tetany	28.8	47.5	6.9	12.9

PROTOCOLS—*Continued*

DOG NUMBER	SEX	WEIGHT	TIME	REMARKS	UREA N	N.P.N.	Ca	P
		kgm.						
363	♀	10	9:45 a.m.		17.1	39.8	11.9	
			10:05 a.m.	30 cc. 5 per cent $CaCl_2$ injected				
			10:15 a.m.	20 cc. 20 per cent NaH_2PO_4 injected				
			10:25 a.m.		15.4		19.6	
			11:15 a.m.		20.4	37.8	20.0	
			11:25 a.m.	20 cc. 5 per cent $CaCl_2$ injected				
			11:30 a.m.	10 cc. 20 per cent NaH_2PO_4 injected				
			12:50 p.m.		19.0	37.0	16.9	
			1:50 p.m.	Collapse. Blood very viscous	23.8	55.2	15.2	
			Post-mortem: Stomach, intestine and spleen congested but non-hemorrhagic.					
350		17						
			6:00 p.m.					3.3
			6:00 p.m.	20 cc. 10 per cent $CaCl_2$ injected				
			10:40 a.m.					3.9
			10:45 a.m.	20 cc. 10 per cent $CaCl_2$ injected				
			12:50 p.m.	Vomiting				4.2
			1:00 p.m.	20 cc. 10 per cent $CaCl_2$ injected				
			1:15 p.m.	Sudden death			17.8	
351		19.5	6:00 p.m.					8.3
			6:00 p.m.	20 cc. 10 per cent $CaCl_2$ injected				
			9:30 a.m.					5.0
			9:30 a.m.	14 cc. 10 per cent $CaCl_2$ injected				
			12:40 p.m.				12.7	4.0
			12:45 p.m.	15 cc. 10 per cent $CaCl_2$ injected Vomited during injection				
			3:00 p.m.				15.2	3.9
			6:00 p.m.	15 cc. 10 per cent $CaCl_2$ injected				
			10:30 a.m.	15 cc. 10 per cent $CaCl_2$ injected				
			1:10 p.m.	10 cc. 10 per cent $CaCl_2$ injected				
			3:40 p.m.				15.9	4.4
			3:45 p.m.	16 cc. 10 per cent $CaCl_2$ injected				
			6:00 p.m.	Fed meat				
			12:30 p.m.	15 cc. 10 per cent $CaCl_2$ injected				
			12:35 p.m.	25 cc. 20 per cent NaH_2PO_4 injected				
			4:15 p.m.	Died			15.1	10.1

PROTOCOLS—*Continued*

DOG NUMBER	SEX	WEIGHT	TIME	REMARKS	UREA N	N.P.N.	Ca	P
		kgm.						
364	♂	20	10:20 a.m.	200 mgm. morphia				
			11:00 a.m.					5.0
			11:00–11:45 a.m.	40 cc. 5 per cent $CaCl_2$ injected				
			1:00 p.m.				19.3	8.7
			1:00–1:10 p.m.	20 cc. 5 per cent $CaCl_2$ injected				
			2:10–2:20 p.m.	20 cc. 5 per cent $CaCl_2$ injected				
			2:20–2:45 p.m.	10 cc. 5 per cent $CaCl_2$ injected				
			3:00 p.m.				24.2	8.0
			3:25–3:50 p.m.	20 cc. 5 per cent $CaCl_2$ injected				
			5:05–5:10 p.m.	10 cc. 5 per cent $CaCl_2$ injected				
			5:35 p.m.					6.6
			8:30 p.m.				19.5	7.7
			8:30–9:30 p.m.	20 cc. 5 per cent $CaCl_2$ injected				
			9:30–9:50 p.m.	15 cc. 5 per cent $CaCl_2$ injected				
			10:30 p.m.	Viscosity increased				6.9
			10:40–11:00 p.m.	10 cc. 5 per cent $CaCl_2$ injected				
			12:00–12:10 a.m.	10 cc. 5 per cent $CaCl_2$ injected				
			12:40 a.m.	Considerable urine during night			27.0	7.1
			10:55 a.m.	Viscosity increased			14.2	5.4
			2:15 p.m.	15 cc. 5 per cent $CaCl_2$ injected				
			2:30 p.m.	300 cc. 0.9 per cent NaCl injected				
			2:45 p.m.	30 cc. 5 per cent $CaCl_2$ injected				
			3:10 p.m.	Tetanic convulsion. Respiratory failure. Death				
			Post-mortem: Negative.					
370	♂	8	10:15 a.m.	80 mgm. morphia				
			11:00 a.m.		13.4	26.6	10.7	
			11:10 a.m.	25 cc. 20 per cent NaH_2PO_4 injected				
			12:00 a.m.					26.0
			1:45 p.m.				7.9	
			2:00 p.m.	15 cc. 20 per cent NaH_2PO_4 injected				
			4:40 p.m.				7.1	8.9
			4:45 p.m.	10 cc. 20 per cent NaH_2PO_4 injected				
			6:30 p.m.	Mild tetany				
			6:30 p.m.	5 cc. 5 per cent $CaCl_2$ injected				

PROTOCOLS—*Concluded*

DOG NUMBER	SEX	WEIGHT	TIME	REMARKS	UREA N	N.P.N.	Ca	P
		kgm.						
370	♂	8	9:20 p.m.	15 cc. 20 per cent NaH_2PO_4 injected				
			11:30 p.m.	40 mgm. morphia				
			10:00 a.m.	In excellent condition	20.4	35.8	10.9	6.2
369	♂	17	10:10 a.m.	170 mgm. morphia				
			11:20 a.m.		13.2	30.8	10.6	
			11:30 a.m.	31 cc. 20 per cent NaH_2PO_4 injected				
			12:00 a.m.					20.0
			2:00 p.m.				9.8	10.0
			2:10 p.m.	24 cc. 20 per cent NaH_2PO_4 injected				
			4:40 p.m.				9.5	12.5
			5:50 p.m.	15 cc. 20 per cent NaH_2PO_4 injected				
			6:30 p.m.	20 cc. 20 per cent NaH_2PO_4 injected				
			9:30 p.m.	20 cc. 20 per cent NaH_2PO_4 injected				
			10:30 p.m.	20 cc. 20 per cent NaH_2PO_4 injected				
			11:30 p.m.	10 cc. 20 per cent NaH_2PO_4 injected				
			11:30 p.m.	45 mgm. morphia				
			10:00 a.m.	In excellent condition	17.9	37.8	11.4	5.2

THE SITE OF REABSORPTION IN THE KIDNEY TUBULE OF NECTURUS

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Received for publication December 23, 1925

The problem of the nature of the fluid in the glomerular capsule was first attacked by direct methods by Wearn and Richards (1924) who showed that the fluid withdrawn from the capsule of the frog was protein-free, contained sugar at a time when the bladder urine was sugar-free and had a chloride content considerably greater than that of the urine. One of their conclusions is that their work "proves the assumption which was made by the earliest of the modern students of renal physiology, that a protein-free, watery fluid is separated from the blood stream as it passes through the glomerular capillaries." An objection to the finality of this proof has been considered in an earlier publication (White and Schmitt, 1925). One fact which is incontrovertibly demonstrated by Wearn and Richards is that somewhere in the tubular system certain of the constituents present in the capsular fluid are reabsorbed. The authors conclude that their experiments "may be regarded as evidence in favor of the filtration hypothesis" and that they "add materially to the evidence advanced by others which indicates that it is not necessary to conceive of a vital secretory process which takes place in the cells of the tubules," but recognize that the evidence on neither of these points is conclusive.

Observations made in this laboratory on the frog's kidney prepared according to Richard's method led us to the conclusion that this kidney was probably not the best material available for the method of micromanipulation. The small size of the capsules, the fact that they are imbedded in the kidney substance, the complexity of the tubular architecture, these are some of the factors which make the application of this method in the frog impracticable for the more complete solution of some of the problems of glomerular and tubular function. We therefore turned to forms which have a kidney better adapted to our purposes. One of the forms used, Cryptobranchus alleghaniensis, has large glomerular capsules but their position deep in the kidney substance offered the same objection as in the frog. Necturus maculosus was found to have a kidney eminently fitted to our needs. There is in this form a longitudinal row of glomerular capsules lying just beneath the peritoneal covering of the ventral aspect of

the kidney near its medial border. These capsules are pear shaped, their transverse diameter averaging 0.5 to 0.8 mm. at this time of year (November and December).[1] Another great advantage of this form, besides the large size and superficial situation of these capsules, is the simplicity of the architecture of the tubules leading from these capsules and the ease, due largely to their superficial location, with which they can be followed and manipulated. A detailed description of the kidney of this form, which has been of great assistance to us in this work, has been published by Chase (1923).

METHODS. In our earlier experiments the animals were anesthetized by immersion in 0.3 per cent urethane previous to pithing. The present procedure is to immerse the animal in 0.2 per cent paraldehyde solution for a few minutes, pith the brain and replace the animal in cold running water for one-half to one hour. It is then fastened to a board and a longitudinal incision made in the ventral body wall about 1 cm. on each side of the midline, bleeding points being cauterized with a hot wire. A ligature is passed from each extremity of one incision through the body cavity to the corresponding extremity of the opposite incision, the ligatures are tied and the ligated area of body wall excised, leaving a window through which the contents of the lower body cavity are seen. The testis or ovary is laid toward the midline and the ventral surface of the kidney thus freely exposed without any traction on the renal vessels. The preparation is covered, except for the exposed area, with gauze soaked in cold tap water and is drenched frequently with tap water; the exposed kidney surface is wet frequently with a stream of Ringer's solution from a wash bottle. The preparation is placed on the stage of a binocular dissecting microscope and illuminated directly from a carbon arc, the light passing through a dilute solution of copper sulphate. In a satisfactory preparation the corpuscles can be seen moving in a lively stream through the glomerular capillaries. We have not found, as did Wearn and Richards, that the introduction of the pipette into the lumen of a capsule brings about a temporary cessation of capillary flow in the glomerulus. We feel that in view of the slow rate at which their fluid was drawn into the pipette and of the various

[1] We wish to call attention here to an error in our calculation in a previous publication (White and Schmitt, 1925) of the capacity of these capsules. It was stated that with an average transverse diameter of 1 mm. the capacity was 3 to 4 cu. mm. On the assumption that a capsule with a transverse diameter of 1 mm. has the same capacity as a sphere of the same diameter, which is probably not far from correct, it is seen to have a capacity of 0.5 cu. mm. From this must of course be subtracted the volume of the tuft which, from many observations, we estimate to be from one-fifth to one-third the volume of a capsule which is functionally active. The average diameter in the winter is less than in the spring; diameters of 0.5 to 0.8 mm. mean capacities of 0.07 to 0.27 cu. mm. respectively, exclusive of the volume of the capillary tuft.

extraneous factors that influence the rate of rise of fluid in the pipette their observation of a cessation of rise of fluid for the few seconds to two minutes during which glomerular capillary flow was checked hardly justifies their conclusion that this observation "indicates. . . . that the fluid drawn from the glomerulus in these experiments did not originate in the tubule."

Glass pipettes were used, those for tubular catheterization having an inside diameter at the tip of 15 to 20μ while those used for capsule work ranged up to 50μ. The pipette is inserted into a heavy-walled rubber tube about 4 cm. long which is connected to a glass leveling bulb by a glass adaptor and a longer segment of rubber tubing, the entire system being filled with mercury. When the bulb is raised 20 or 30 cm. the mercury column comes almost to the tip of the pipette, the distance by which it fails to reach the tip varying with the inside diameter of the terminal portion of the pipette. A screw clamp is on the heavy-walled rubber tube as close as possible to the end of the pipette fitting into it. Another screw clamp is on the longer segment of tubing just beyond the glass adaptor. Accurate control of the mercury column, which is essential to a safe delivery of the collected fluid into the capillary tubes where it is to be analyzed, is effected by the following procedure. With both screw clamps open the leveling bulb is raised 20 to 30 cm., bringing the mercury column almost to the tip of the pipette. The proximal (with respect to the pipette) clamp is then screwed down until it is almost but not quite closed; the distal clamp is then closed. Completion of the closing of the proximal clamp then moves the mercury column under perfect control out to the very tip of the pipette preliminary to puncture. When the puncture has been made opening of the proximal clamp draws the fluid into the pipette Application of negative pressure may continue as long as needed by lowering the leveling bulb and opening the distal clamp. When sufficient fluid has been obtained the system is put under atmospheric pressure, the distal clamp closed and the pipette withdrawn. The tip of the glass capillary receiving tube, with an inside diameter of 75 to 150μ, which is held on a Zimmerman stand, is then brought into the field of the microscope, the tip of the pipette inserted into it and the fluid expelled by closing the proximal clamp. We have found it very difficult to control the movement of the fluid column with sufficient accuracy to enable us to deliver any desired portion into the receiving capillary by merely applying a positive pressure by raising the leveling bulb. Various schemes for ensuring an accurate control of the mercury column were tried before the one described above was adopted. One of these was to have the entire mercury system in an apparatus with rigid walls, the pipette being joined to a thin-walled steel cylinder by a flexible metal tubing sealed on with deKhotinsky cement. Movement of the column was brought about by a clamp squeezing on the

walls of the steel cylinder. Although the movements of the mercury column can be very accurately controlled in this way the great difficulty encountered in completely filling the system with mercury rendered it impracticable for repeated use. Another plan tried was to seal a small metal syringe with a metal plunger directly to the glass pipette. We were not able, however, to make the plunger fit tight enough to prevent leaking against the rather high pressure required to force the mercury column out to the tip. In order to get an accurate control of the column with the apparatus finally adopted and described here it is essential that the mercury and glass and rubber tubing be quite clean. Rubber tubing should be boiled for a few minutes in a 1 or 2 per cent sodium hydroxide solution to remove the sulphur. All air bubbles must of course be excluded.

After a quantity of fluid sufficient for the test desired has been expelled into the receiving capillary the reagent with which it is to be mixed is introduced from a pipette similar to the one employed for collecting fluid. The tip of this pipette, which is drawn with a narrow shank, is inserted into the receiving capillary until it enters the column of fluid; an intimate mixture of fluid and reagent is thus ensured. We have had some difficulties in employing the technique described by Wearn and Richards for mixing the fluid and reagent. In the first place, when the capillary is broken at the middle of the fluid column this frequently recedes from each broken end; when this end is then dipped into a drop of reagent there is an air bubble between fluid and reagent which it is often impossible to eliminate and which may cause falsely negative reactions. In the second place, it is impossible to control accurately the proportion of reagent to fluid when the reagent is drawn in by capillarity. We have sometimes found it desirable to control this proportion and by injecting are able to introduce any desired volume of reagent. Sugar is tested for with Benedict's qualitative reagent, chloride with 1 per cent silver nitrate acidified with nitric acid, protein by potassium ferrocyanide-acetic acid or concentrated nitric acid.

In our earlier experiments the pipette to be introduced into capsule or tubule was held in a microdissector of the type described by Chambers (1922).[2] We have later developed a much simpler pipette holder which permits of manipulation sufficiently accurate for our purposes. Whichever type of maniputator is used the tip of the pipette is first brought into the proper horizontal plane practically touching the wall of a capsule or tubule. The animal board is then moved by hand along the microscope stage until the capsular or tubular wall is pushed up against the tip of the pipette, when a quick but accurately controlled push on the board introduces the pipette into the capsule or tubule. The manipulator made in

[2] We wish to acknowledge here our indebtedness to Prof. R. J. Terry for the use of the microdissector.

this laboratory is of quite simple construction. There is a brass holder, *A*, into which the pipette can readily be inserted and fixed by a screw, *I*, which by impinging on the pipette in front of its vertical diameter presses it firmly against the back wall of the holder, *A*. This fits firmly into a

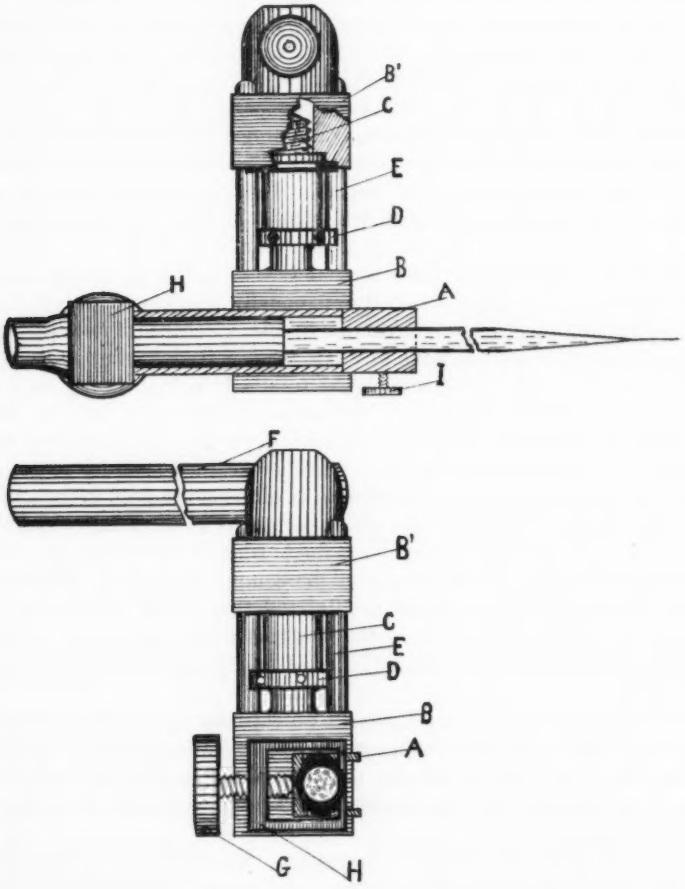

Fig. 1

brass block, *B*, which is raised or lowered by turning the central adjusting screw, *C*. Turning of this screw is facilitated by a lever pin (not shown in illustration) inserted into holes in the rim of the nut, *D*. Lateral movements of the holder are prevented by the steel guide pins, *E*, which are

fastened to the lower block, *B*. The threaded portion of the central adjusting screw, *C*, is rigidly fastened to the lower block, *B*, and fits into the nut, *D*, which is fastened by suitable bearings to the upper block, *B'*. The instrument is clamped to a heavy ring stand by the supporting rod, *F*. Movements of the mercury column in the glass pipette are effected by the screw, *G*, of the clamp, *H*, which fits over the heavy-walled rubber tube into which the pipette is inserted. One side of the holder, *A*, serves as a stationary support against which the rubber tube is compressed. This makes possible adjustments of the screw with one hand. This clamp, *H*, has been termed the proximal clamp in the preceding description of the system.

In the kidney of Necturus we believe we have found conditions almost ideal for the application of this method. It is an astonishingly simple matter to obtain protein-free glomerular fluid in sufficient quantities to test chemically, (we have on one occasion obtained 5 cu. mm. of capsular fluid in thirty minutes), and after some degree of facility of manipulation of the apparatus has been attained it is equally possible to obtain fluid from any part of the proximal convoluted tubule. Because of its superficial distribution it is usually possible to insert the pipette into any part of the tubule desired. The distal convoluted tubule is not so easily entered because of its deeper distribution and smaller lumen. To date we have not obtained fluid from the distal convoluted tubule. It is not essential, however, for our purposes that such fluid be obtained in order to get an idea of the rôle which this segment plays in altering the fluid in its course down the tubule. We have data which permit us to form fairly definite ideas as to the chemical nature of the fluid when it leaves the proximal convoluted tubule. The difference in the composition of the fluid upon leaving the proximal convoluted tubule, and that of the urine may be thought of as being due to the activity of the distal convoluted tubule, assuming that the fluid is unaltered in its passage from the distal tubule to the bladder.

The findings of Wearn and Richards on capsular fluid were confirmed by us in the kidney of Necturus in an earlier publication (1925). The following table will suffice to indicate the type of results obtained with this method, as selected from experiments on Necturi collected at different times of the year.

Localization of the site of sugar reabsorption. The method of determining the part of the tubular system in which the sugar is reabsorbed is very simple. All that need be done is to withdraw fluid from various parts of the tubule and test for sugar. If the test is negative for a certain point the sugar must have been reabsorbed at some point nearer the glomerulus. This statement requires some qualification. We can state here that at no time did a protein-free fluid obtained from the proximal convoluted tubule ever give a positive sugar test. This does not necessarily mean,

however, that the sugar was reabsorbed in the proximal tubule for Necturi collected in the late fall and winter often have blood sugars of such low concentration as to render quantitative determination impossible. Accordingly it is necessary to show that there is sugar present in the capsule at a time when sugar is absent in the fluid collected from the proximal convoluted tubule in order to localize the site of reabsorption to this part of the tubule.

The complete experiment is done as follows. After the animal is pithed a subcutaneous injection of 2 cc. (for a large specimen) of 1 per cent glucose solution is given. The animal is returned to cold flowing tap water for a half to one hour, when it is prepared as described above, a capsule punctured and the protein-free fluid tested for sugar. If no sugar is found, more time is allowed for absorption of the injected sugar

TABLE 1

NUMBER OF EXPERIMENT	DATE	PLASMA SUGAR	SUGAR IN		
			Capsular fluid	Proximal tubule fluid	Urine
		per cent			
1	May 15	0.08	++		0
5	May 20	0.08	++		0
9	November 14			0	0
12	November 17			0	0
13	November 25	0.035*	++		0
14	November 25	0.035	++	0	0
15	November 28	0.046†	++	0	0

* First capsular fluid obtained was sugar-free. One cubic centimeter of 5 per cent sugar solution was injected into the gut. This raised the blood sugar to the value shown which was sufficient to demonstrate sugar in the capsule.

† Two cubic centimeters of 1 per cent sugar solution injected subcutaneously immediately after pithing.

or the animal is discarded for the purposes of this experiment. If the capsular fluid contains sugar, preparations are made for a tubular puncture.

The first tubular punctures tried were made as close as possible to the distal end of the proximal convoluted tubule. A fine pipette is inserted into the tubule so that the angle which the pipette makes with the plane of the tubule is as small as possible. This helps prevent the stopping of the aperture of the pipette by contact with the inner tubule wall and facilitates the withdrawal of fluid. The pipette is never left in the tubule for more than ten to fifteen minutes. If the circulation is active and the tubule fairly well distended with fluid it is easily possible to get from 0.05 to 0.1 mm. of fluid, an amount ample for a single sugar test. We feel that the possibilities of disturbing cell function are reduced to the minimum by the short stay of the pipette in the tubule.

Fluid from the far end of the proximal convoluted tubule was found to be sugar-free at a time when the capsule fluid contained sugar. This proves that all the sugar which found its way into the capsule was reabsorbed in its passage down the proximal convoluted tubule or at least that the concentration was reduced to the point where it failed to respond to Benedict's test. We next attempted to see how close to the capsule the fluid could be withdrawn and still be sugar-free. The proximal tubule was entered at points such that approximately one-half and one-third the proximal tubular length was between the site of puncture and the capsule. Since the proximal tubule proceeds laterally from the capsule to the edge of the kidney and since the opening of the pipette as it enters the tubular lumen is looking towards the glomerulus, by regulating the size of the pipette and the manner in which it is inserted into the tubule one may be reasonably certain that the fluid withdrawn has been acted on only by that part of the tubule between the capsule and the tip of the pipette.

Fluids obtained from a point halfway down the proximal tubule are consistently sugar free. We have made one puncture very close to the capsule at a point between one-third and one-fourth the way down the tubule. This fluid also proved to be sugar-free. In this way we were able to demonstrate that practically all the sugar which enters the capsule is reabsorbed certainly by the time it has traversed half the proximal tubule. This fact would indicate a very high efficiency of the proximal tubule for reabsorbing sugar.

Localization of the site of chloride reabsorption. That chlorides are reabsorbed somewhere in their passage down the tubules has been clearly demonstrated by the experiments of Wearn and Richards, by perfusion experiments on the isolated kidney by Starling and Verney (1925), as well as by our earlier experiments on the composition of the capsular fluid (1925). Until now, however, it has been obviously impossible to localize the site of this reabsorption.

Haldane, Peskett and Davies (1922) and Starling and Verney (1925) by indirect reasoning from conditions which obtained in their experiments were able to designate roughly the point of reabsorption of various substances relative to each other. From their experiments on the perfused kidney in urea diuresis, Starling and Verney were led to believe that chlorides were reabsorbed at some point higher up in the tubular system than the point at which water is reabsorbed. Such results must be regarded, however, as being only hypothetical. Although their reasoning would tend to indicate the point of reabsorption of chloride, urea and water relative to each other, they fail to definitely localize the site of their reabsorption as none of these has ever been fixed individually. Such a localization is obviously impossible unless the tubule can be entered and the composition of the fluid at various points tested.

It was brought out in our preliminary note (1925), that most of the chlorides which enter the glomerular capsule of Necturus are reabsorbed by the tubular system. There is, however, a definite concentration of chlorides consistently present in the urine. The obvious method of localizing the site of chloride reabsorption in Necturus would be similar to that of determining the site of sugar reabsorption, namely, by testing various tubular fluids for chlorides. Since a definite amount of chlorides normally get through without reabsorption, conclusions could be drawn only by quantitative determination of the chloride concentration of the fluid from these various segments.

The difficulty of such a method will become apparent upon consideration of the minute quantities of fluid available for analysis. The average yield of fluid from a brief period of collection from the proximal tubule is about 0.1 cu. mm. With sufficient care a series of tubes of the same bore could be prepared in which equal quantities of silver nitrate and chloride solutions of concentrations ranging from that which obtains in the urine to that of the blood could be mixed. The precipitates in these tubes could be used as standards with which to compare the precipitate obtained in a similar tube in which tubular fluid could be mixed with an equal volume of silver nitrate. This was tried but found to be only very roughly quantitative and very cumbersome to use.

It seems a safe assumption that that point in the tubular system at which the osmotic pressure of the fluid markedly drops should mark the site of chloride reabsorption. If, then, we had some means of following the osmotic pressure changes in the different segments of the glomerulo-tubule system, we should be in a position to localize chloride reabsorption.

It occurred to us that possibly some form of small free cell which would exhibit some definite morphological change in the range of osmotic pressures approximating those in the glomerulo-tubule system, and which would at the same time be plainly visible through the cellular mass of the tubule, would be an ideal indicator of this difference in pressure.

All these conditions are admirably met in dog red blood corpuscles stained with methylene blue. Freshly drawn blood is centrifugated and the corpuscles washed three times in 0.7 per cent Ringer's solution. These washed cells, suspended in Ringer's solution are then stained by shaking them in filtered methylene blue solution of a concentration of about one to five thousand. It is preferable to allow these cells to remain in the dye for at least an hour to ensure their adequate staining.

It was found that the cells thus stained were practically unaffected in 0.5 per cent salt solution, were practically hemolyzed in 0.3 per cent salt solution and completely hemolyzed in 0.2 per cent salt solution. The staining increases the resistance of these cells to laking. Unstained cells are unaffected in 0.6 per cent salt solution but completely hemolyze in 0.3 per cent salt solution.

The experiment proceeds in the following manner. A pipette is drawn with a fairly large aperture (50μ). A small droplet of the concentrated suspension of washed and stained cells is put on the edge of a piece of glass, the tip of the pipette dipped into the droplet and about five to ten cubic millimeters of cells drawn into the pipette. The animal whose kidney has been prepared is then placed under the microscope. After an examination of the kidney, the most superficial, and at the same time the most nearly transparent set of tubules is selected for injection. In most cases the capsule from which the selected tubular system arises can be determined. The pipette is then inserted into this capsule in a way such as to prevent any tearing of the membrane, and the contents of the pipette injected into the capsule until the entire tubular system is seen to be filled with the blue stain. Quickly withdrawing the pipette, the high power objective is put in place and all parts of the system observed. The tubules which contain the blue stain are closely watched for the presence of cells. At no time can any cells be discerned in any part of the tubular system beyond the narrow neck connecting the capsule with proximal tubule, although the capsule itself is filled with them.

The individual cells can easily be distinguished in the capsule and their number is seen to be constantly diminishing. That this is due to the fact that the vigorous circulation is producing more glomerular fluid and washing these cells down into the tubules where they are almost instantaneously laked is proven by the following experiment. A glomerular puncture is performed, capsular fluid collected in the pipette, and the pipette withdrawn from the capsule. The pipette is then dipped below the surface of a droplet of cell suspension and a very small quantity of cells drawn up into the capsular fluid. These cells are observed with the high power objective for some while. No appreciable laking takes place and the majority of cells thus suspended in capsular fluid are unaffected after many minutes. This proves that the cells were not laking in the capsule but were disappearing only because they were washed down the tubule where they were laked and could no longer be seen. The methylene blue is quickly reduced by the tubule cells so that in two to five minutes after the injection the lumen of the tubules is again colorless. Thus the methylene blue serves as a criterion of the viability of the tubule cells as well as an indicator of the course of the tubules injected with cells.

It may be supposed that the reason that no cells were seen in the tubules when they were quite apparent in the capsule is that in the former case the thickness of the tubule cells makes close observation of the tubule contents impossible while in the case of the capsule only a thin membrane obstructs the view. To prove that this was not the case the following experiment was performed. A quantity of washed red cells was fixed in 10 per cent formalin solution for several hours. The cells were separated by centrifu-

galization and washed in Ringer's solution. To a suspension of these cells was added some methylene blue of a concentration of about one to five thousand in Ringer's solution. It is preferable to allow these fixed cells to remain in the stain overnight as the process of staining after fixation is a slow one. The resulting cells suffer no appreciable change when placed in distilled water.

The fixed and stained cells are then separated from the dye by centrifugalization and washed twice in Ringer's solution. A heavy suspension of these stained cells is made and drawn up into a pipette and injected into a capsule. The entire tubular system is beautifully outlined by the presence of the stained cells in the lumen. Each individual cell can be easily distinguished and they undergo no change in the tubule except that they are washed down by the stream of fluid from the capsule. This furnishes proof positive that if the living cells had not been laking in the tubule in the preceding experiment they would have easily been detected. This was further strikingly corroborated by the fact that if a suspension of Necturus red cells, which do not lake in distilled water, be injected they can easily be seen in the tubule even though unstained.

It may be further supposed that possibly our failure in seeing the cells in the tubule could be due to the passing out of the dye before hemolysis. That this is not the case is proven by the observation that no dye passes from cells into a salt solution, the osmotic pressure of which is such that hemolysis does not take place. A further objection might be raised on the supposition that the dye in the cells was being reduced, making their detection difficult. But no cells were seen even while the fluid in the tubular lumen was still blue. There is no reason to suppose that the dye in the cells would be reduced sooner than that in the surrounding fluid. Furthermore, no reduction of the dye in unfixed, stained cells of Necturus, which do not lake in tubular fluid, is observed even after a long stay in the tubule.

Data on blood and urine chemistry. It has seemed desirable to obtain some data on the blood and urine chemistry of the form used in the present work. Our animals were caught at Ithaca, New York. The first group was caught in April, 1925, and these animals had blood sugar values ranging from 60 to 80 mgm. per 100 cc., by the Shaffer-Hartman method. Only blood sugar and urine chloride determinations were carried out in this first group of animals. The sugar figures obtained on the five bloods examined are 70, 60, 80, 70 and 60 mgm. per 100 cc., the chloride figures on two urines 20 and 17 mgm. per 100 cc. All urines have been protein- and sugar-free.

The following table gives the data obtained on the blood and urine of animals caught this fall, November, 1925. No sugar was administered to these animals.

It will be noted that in only one of the four bloods examined for sugar in the November group was there any significant amount. The zero given in the table as the blood sugar value in the other three animals means that the difference between the thiosulphate titration figures of blank and tungstic acid filtrate was less than 0.5 cc. (0.2, 0 and 0.3 cc.). It is recognized that the sugar values on filtrates with a titration figure of less than 0.5 cc. are not of quantitative significance. The zero figure values are thus to be interpreted as meaning that the total reducing substances in these bloods

TABLE 2

Chemical data on urine, blood and plasma of untreated November animals

	NaCl PER 100 CC.	INORGANIC PHOSPHATE P PER 100 CC.	UREA N PER 100 CC.	GLUCOSE PER 100 CC.	NON-PROTEIN NITROGEN PER 100 CC.	VOLUME PER CENT OF CELLS IN BLOOD
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
Urine	26	5.4	12[3]	0[6]	11[2]	
	21	10.0[4]		0[7]		
		2.5		0[8]		
		14.0				
		trace[5]				
		11.0				
Blood	280	7	7.4[3]	50[6]		33
	258	8		0[7]		32
						28
						30[1]
						29
Plasma	360[1]	5.1[1]		0[8]	7.6[2]	
	365	5.8		0		
	358	5.4[4]				
	348	4.8				
	358	5.6[5]				

When more than one constituent has been determined on one animal the figures obtained on a given animal are designated by the small figures, 1, 2, etc. Thus the data designated by the small figure 1 show that one animal had 360 mgm. NaCl per 100 cc. plasma, 5.1 mgm. inorganic P per 100 cc. plasma and 30 volume per cent cells in the blood.

or plasmas are less than the equivalent of 20 mgm. of glucose per 100 cc. of blood, which means that they would fail to give a positive test with Benedict's qualitative reagent. This animal is seen to conserve chloride almost as efficiently as sugar. The urine is very low in total solids, including nitrogen. This may be due to the low metabolic state of this form in the early winter. We expect to make further observations on the phosphate and nitrogen output in their active state in the spring.

SUMMARY

The advantages of Necturus maculosus as a form for micromanipulation of the kidney are stated.

The technique of withdrawal, delivery and analysis of capsular and tubular fluid is described.

A pipette holder suitable for this procedure is described and illustrated.

Sugar is reabsorbed in the proximal convoluted tubule. This is demonstrated by the fact that capsular fluid contains sugar at a time when fluid gotten from the tubule is sugar-free.

Chlorides are reabsorbed in the proximal convoluted tubule. This is demonstrated by the fact that red cells injected into the glomerulo-tubule system lake in the tubular fluid at a time when they are unaffected in the capsular fluid.

Chemical data on the blood and urine of this form are presented.

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THE AMERICAN JOURNAL OF PHYSIOLOGY is issued monthly by the American Physiological Society under the direction of the Council of the Society. From three to four volumes, each of about six hundred and fifty pages, are published yearly. The subscription price per volume in the United States and Canada is $5.00; in other countries, $5.25.

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